Gerard L. Hasenhuettl
Richard W. Hartel *Editors*

Food Emulsifiers and Their Applications

Third Edition

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Preface to the Third Edition

Next to water, food is the most critical resource to ensure life. The gold standard for good food has been an appetizing meal, prepared from fresh ingredients. Unfortunately, ingredients do not remain fresh for long. The quality of prepared foods is likewise fleeting. Packaged foods have been developed to fill in when fresh ingredients are unavailable, or convenience is essential.

Food additives were developed in the twentieth century to improve the stability, texture, and flavor of foods. Food emulsifiers have been classically defined as ingredients that promote the formation of or maintain the stability of emulsions. The definition may be generalized further to mixing of insoluble phases. Foams are mixtures of gas and liquid, and dispersions are mixtures of liquids and solids. The action of these ingredients occurs at an interface or surface. For this reason, the terms emulsifier and surfactant have been used interchangeably.

The first emulsifiers were naturally occurring ingredients, such as egg or casein. Advances in chemistry and engineering led to synthesis of surfactants that produced superior and more economical effects. The wide selection of food emulsifiers has led to wide geographical distribution of packaged foods. This capability improves scale economies, which allows more economical pricing for consumers. Lecithin, egg yolk and white proteins, and protein-containing hydrocolloids are naturally occurring emulsifiers, with long histories of use in food applications. Other surfactants are produced by chemical reaction of fats, oils, or fatty acids with other well-known biological molecules. Emulsifiers as well as other food additives are regulated by government agencies, such as the U.S. Food and Drug Administration and the European Economic Community.

Surfactants are amphiphilic molecules, having one or more hydrophobic regions, and one or more functional groups. They promote and stabilize emulsions by orienting themselves at the oil-water interface and lowering the interfacial tension. Interactions with other food components, such as water, carbohydrates, and proteins, are important to promote other functions. Mechanisms of these interactions are discussed in Chaps. 4, 5, and 6.

Applications in food products are discussed in Chaps. 7–16. Chapters describing dressings and sauces and processed meats are new in the Third Edition.

Consumers have favored natural foods and have resisted chemical additives. Affluent consumers have been willing and able to pay the higher cost for these foods. Lower income consumers may attempt to grow their own food, but are limited to growing seasons and access to arable land. The challenge for processed food manufacturers is to produce nutritious foods having good flavor and texture with minimal chemical additives. Scientists, product developers, engineers, and students may find this book useful for addressing this challenge.

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Chapter 1
Overview of Food Emulsifiers

Gerard L. Hasenhuettl

1.1 Introduction

Spoilage has been a serious problem throughout history. Innovations in processing, preservation and packaging have dramatically increased the capability to distribute food around the world.

Many processed packaged foods contain mixtures of mutually insoluble phases, such as gas, liquid, and solids. Table 1.1 shows examples of these foods. Mixtures of insoluble phases are thermo dynamically unstable, and will eventually separate. Separation of these phases can cause problems with texture and appearance, flavor, and microbial stasis. For example, a creamy salad dressing may turn from white to gray, after storage on a shelf for a few months. Food emulsifiers can help to retard separation. A competing approach is to increase the viscosity of the continuous phase, in order to reduce mobility of dispersed phase (particles, droplets, or bubbles). For water-continuous products, starches and gums (also referred to as hydrocolloids) can exert this thickening effect. Additives for oil-continuous systems are solid fats, waxes, and calcium salts.

Emulsifiers may be defined as additives that promote the formation, or enhance the stability of emulsions. Food emulsifiers often provide technical benefits, such as viscosity reduction, starch complexation, or inhibition of crystallization. These additives may be more correctly defined as surfactants. Because of widespread usage of the term emulsifier in the literature, it will be preserved in this book, as an interchangeable term with surfactant.

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Table 1.1 Multiple phase mixtures in food products

	Continuous phase			
	Phases	Solid	Liquid	Gas
Dispersed phase	Solid	Alloy/solid solution Chocolate	Dispersion Chocolate beverage	Solid aerosol Spray icings
	Liquid	Semi-solid emulsion Margarine	Emulsion Creamy dressing	Liquid aerosol Spray oil
	Gas	Foam Whipped topping	Gas dispersion Carbonated beverage	Gas mixture Air

A detailed discussion of food emulsions, dispersions, and foams is far beyond the scope of this book. There are several excellent sources in the literature (McClements 2004; Sjobiom 2013; Phillips 2009).

The earliest known emulsions were based on natural occurring materials. Milk, for example, is a dispersion of fat droplets in an aqueous media, stabilized by milk proteins and phospholipids. Early formulations for butter, cheese, and whipped cream took advantage of these naturally occurring emulsifiers. The invention of mayonnaise in France dispersed oil into an acidified aqueous phase, using egg yolk to stabilize the emulsion. The functionality of egg lipoproteins is still impressive by today's standards. Up to 80% oil can be emulsified without inversion to a water-in-oil emulsion.

In 1889, French chemist Hippolyte Mege-Mouries invented margarine as a low-cost substitute for butter. He dispersed an aqueous phase into molten tallow, followed by cooling. In this case, stability is maintained by restricted motion of water droplets by the solid tallow phase. Discovery of the hydrogenation process allowed semi-solid vegetable fat to be substituted for the tallow. Synthetic food emulsifiers were developed during the second half of the Twentieth Century, in response to the need for shelf-stable foods for mass market distribution. For example, creamy salad dressing can now remain stable for a year without phase separation. Rancidity and flavor degradation are now the factors which limit shelf life. Synthetic food emulsifiers are less expensive than gums, but bear the stigma of chemical-sounding names.

Food emulsifiers are amphiphilic molecules, having a hydrophilic (water loving) head group and a lipophilic (oil or lipoid loving tail. The lipophilic tails are even-numbered straight-chain fatty acids. They are derived from animal or vegetable fats and oils. Hydrophilic groups are ionic or dipolar. They are derived from molecules that are edible. Proteins can also be surface-active if they contain both hydrophilic and lipophilic amino acid residues. Figure 1.1 shows how emulsifiers are oriented at the oil-water interface.

Synthetic food emulsifiers may be produced as liquid, solid, or semi-solid physical states.

Liquids are packaged in buckets, drums, or may be delivered in tank trucks or rail cars. Solids are flaked or spray-chilled into beads, and delivered in plastic-lined cartons or drums. Emulsifiers may also be blended with stabilizers or other ingredients for convenient addition of the customer.

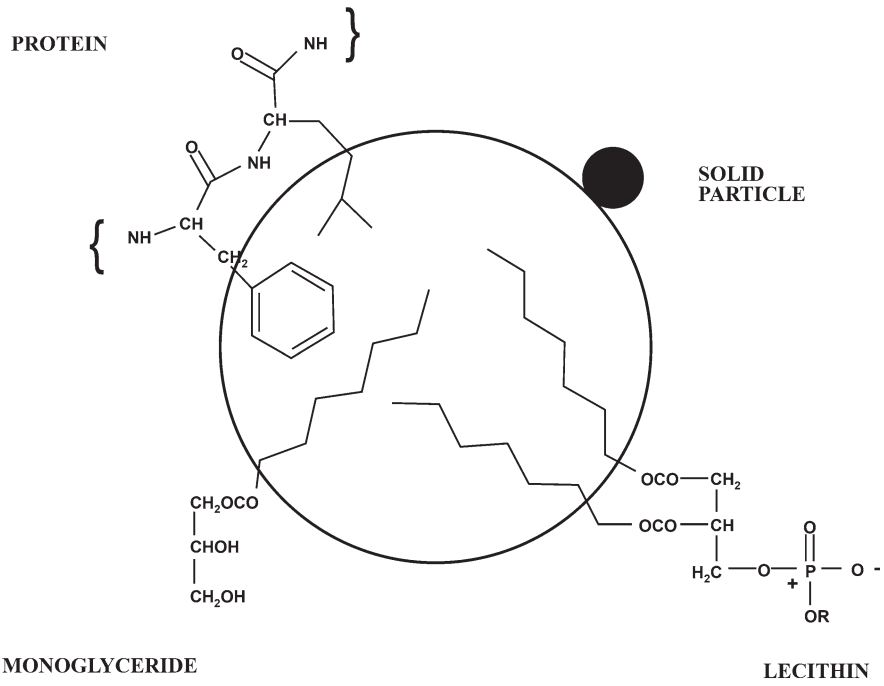

Fig. 1.1 Orientation of surfactants at the oil/water interface

1.2 Food Additive Regulations

Food emulsifiers are regulated as food additives in almost all major countries around the world. In the United States, the Food and Drug Administration (Federal Register 2013) regulates the composition of foods, drugs and cosmetics. Food regulations may be found on Title 21 of the Code of Federal Regulations (21 CFR). The most lightly regulated additives are listed in Section 182, Generally Recognized as Safe (GRAS), and Section 184, Affirmed as GRAS. These substances have long histories of use and clean safety records. Table 1.2 lists these emulsifiers. They may be used in foods as long as they are not precluded by a standard of identity.

Other emulsifiers are listed as Direct Food Additives in Section 172. Here the restrictions vary widely. Some emulsifiers are allowed at levels "sufficient to accomplish the desired technical effect." Others are restricted for methods of manufacture, analytical measurements, specified food products, and use levels. Table 1.3 lists these emulsifiers. Companies that produce emulsifiers can petition the FDA for approval of a new food additive, but documentation of safety is expensive and time consuming. It is less burdensome to obtain permission for expanded use for an additive currently approved for a different product, or an additive with a history of safe use in another country. The process, however, still takes several years.

Table 1.2 Food emulsifiers affirmed as GRAS

Emulsifier	U.S. FDA (2ICFR)	EEC (E no__.)
Diacetyltartaric ester of Monoglycerides (DATEM)	184.1101	E472e
Lecithin	184.1400	E322
Mono- and diglycerides	184.1505	E471
Monosodium phosphate derivatives of mono and diglycerides	184.1521	–

Table 1.3 Emulsifiers listed as direct food additives

Emulsifier	U.S._FDA_(21CFR)	E EC (E No.___)
Acetylated mono- and diglycerides	172.828	E472a
Calcium stearoyl lactylate	172.844	E482
Citric acid esters of mono- and diglycerides	172.832	E472c
Ethoxylated mono- and diglycerides	172.834	–
Lactic acid esters of mono-and diglycerides	172.850	E472b
Magnesium salts of fatty acids	172.863	–
Polyglycerol polyricinoleate	–	E476
Polysorbate 60	172.836	–
Polysorbate 65	172.838	–
Polysorbate 80	172.840	–
Propylene glycol esters of fatty acids	172.856	E477
Salts of fatty acids	172.863	E470a
Sodium stearoyl lactylate	172.846	E481
Sodium stearoyl fumarate	172.826	–
Sorbitan monolaurate	–	E493
Sorbitan monooleate	–	E494
Sorbitan monopalmitate	–	E495
Sorbitan monostearate	172.842	E491
Sorbitan tristerate	–	E492
Stearyl tartrate	–	E483
Succinylated mono-and diglycerides	172.830	–
Sucrose acetate isobutyrate (SAIB)	172.833	–
Sucrose esters of fatty acids	172.859	E473
Tartaric acid esters of mono-and diglycerides	–	E472d

The European Economic Community (EEC) regulations are similar to those in the United States. E-numbers for the commonly used emulsifiers are shown in Tables 1.2 and 1.3. However, when preparing to sell a product in another country, details of the regulations must be examined. For example, polyglycerol esters with a degree of polymerization up to 10 are acceptable in the United States. In EEC countries, the maximum degree of polymerization is 4.

Countries that have not formed trading agreements may have unique food regulations. Translation from the local language may result in misunderstanding. Local consultants or trading partners can provide valuable information and contacts.

In order to reach Jewish and Islamic consumers, many manufacturers require their ingredients to conform to Kosher or Halal requirements. Food emulsifiers must be prepared from kosher certified raw materials, for example, vegetable oils. Manufacturing must also be carried out in a Kosher-certified plant, and not contaminated by non-Kosher materials. The major hurdle to clear is to ensure that the customer's rabbinical council will accept the Heckshire (Kosher symbol) of the supplier's rabbi.

Although standards remain somewhat loose, products that claim to be "all natural" must not contain ingredients that have been chemically processed or modified. Food emulsifiers for these products are limited to lecithin, dairy and egg proteins, surface-active gums, or other naturally occurring materials.

1.3 Food Emulsifier Structure and Function

Emulsifiers are amphiphilic molecules, containing polar and non-polar regions. In most cases, the non-polar units are derived from fatty acid of 16 or more carbon atoms. Shorter chain lengths make excellent emulsifiers, but can react with water to produce soapy or other undesirable off-flavors (hydrolytic rancidity). Unsaturated fatty acids (oleic, linoleic, and linolenic) can react with oxygen to produce cardboard, beany, grassy, or painty off-flavors (oxidative rancidity). Liquid oils may be hydrogenated to produce semi-solid fats, referred to as "plastic."

Polar functional groups are hydroxyl, amino, and carboxylic or phosphoric acids that can be incorporated into the structure to produce anionic (negative charge), cationic (positive charge), amphoteric (positive and negative charges), or nonionic (no formal charge) emulsifiers. Monoacyl glycerols, commonly called monoglycerides, are the most commonly used nonionic surfactants. Calcium and sodium stearoyl lactylates are anionic surfactants widely used in bakery products. Lecithin can function as an amphoteric or cationic surfactant, depending on the pH of the food product.

Proteins can also be surface active if they contain Lipophilic amino acids such as phenylalanine, leucine, and isoleucine. These side groups are attracted to the oil phase, while polar amino acid, such as aspartic or glutamic acids, tend to extend into the aqueous phase. Proteins can form loops around oil droplets and prevent coalescence and separation (steric stabilization). Charged amino acids can stabilize emulsions by electrostatic repulsion of the droplets. However, they can destabilize oil-continuous emulsions, such as low fat spreads, by causing inversion.

Food emulsifiers may be thought of as designer molecules. The size and number of hydrophilic head groups and lipophilic tails can be independently varied. A useful conceptual tool is the hydrophilic-lipophilic balance (HLB). Becher (2001) has extensively reviewed the subject, so it will not be discussed in detail here.

HLB values determine whether hydrophilic or lipophilic groups are dominant. Values for food emulsifiers range from 2 to 16. Low HLB emulsifiers readily dissolve in oil, while high HLB emulsifiers dissolve in water. High HLB emulsifiers are effective emulsifying agents, e.g. for dissolving flavor oils in the aqueous phase. High HLB emulsifiers are useful for preparing oil-in-water (O/W) emulsions, while low HLB emulsifiers are good for water-in-oil (W/O) emulsions. Emulsifiers with intermediate HLB values tend to be poorly soluble in both oil and water, so they tend to accumulate at the interface.

Conventional practices to add the emulsifier to the continuous phase, and then slowly add the dispersed phase with vigorous mixing, this is known as Bancroft's Rule. One exception is to dissolve Polysorbate 60 to the oil phase when preparing a salad dressing. A finer and narrower droplet distribution is achieved.

Surfactants can react with water to form ordered structures called mesophases, sometimes referred to as liquid crystal. The most common of these bilayer systems are lamellar, hexagonal, cubic, and vesicular.

The lamellar phase consists of an infinite bilayer plane where polar head groups are associated with one another. Above the melting point of the alkyl chains (Kraft temperature), large amounts of water can enter the space between the polar head groups. When the system is cooled, the alkyl chains crystallize, trapping the water and reducing its concentration in the continuous phase. The swollen bilayer is called an alpha gel.

The hexagonal phase is an assembly of cylindrical or barrel-like structures. Type I has the fatty acid chains inside the cylinder, with the head groups outside, facing the water-continuous phase. The Type II structure has the reverse arrangement, with the polar head groups inside, and the fatty acid chains protruding into the continuous oil phase.

Cubic phases are complex three-dimensional structures, with single distinct internal and external phases. The tortuous paths inside the cubic phase have recently been investigated for applications in a number of disciplines.

Vesicles, sometimes referred to as liposomes, are spherical bilayer structures. The most common forms are large unilamellar vesicles (LUV) and small unilamellar vesicles (SUV). Vesicles have been explored for controlled release and drug targeting in the pharmaceutical industry.

Israelachvili (1992) has developed a predictive model that relates relative sizes of the lipophilic and hydrophilic groups to the type of mesophase formed when the molecule is dispersed. A critical packing coefficient is calculated by determining the hydrodynamic volume (includes bound water molecules) of the head group and the volume occupied by the lipophilic tail. Figure 1.2 and Table 1.4 shows these relationships. The major limitation of this approach may be the heterogeneous composition of commercial food emulsifiers. This will be discussed further in Chap. 2.

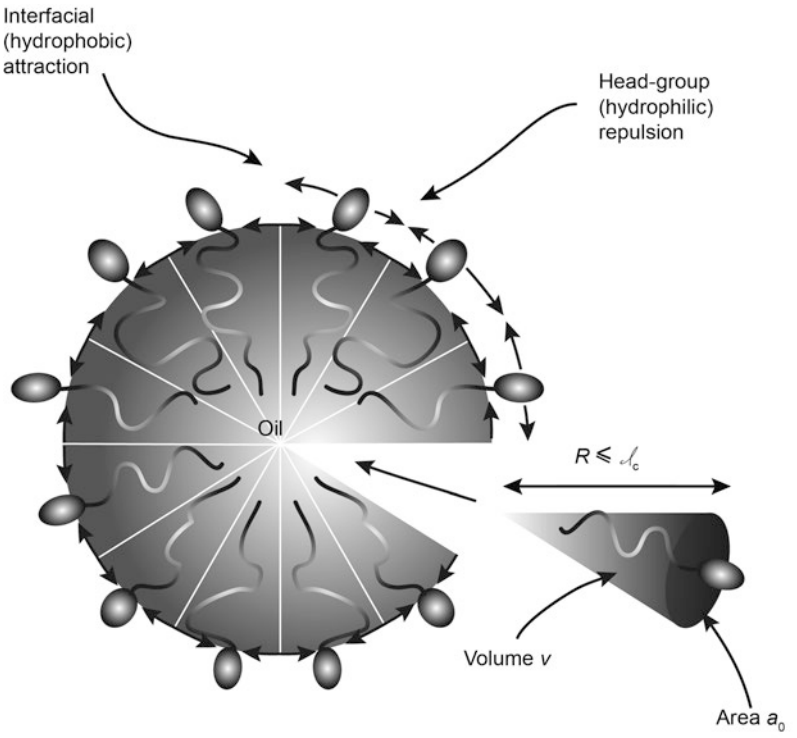

Fig. 1.2 Determination of the critical packing parameter (Israelachvili 1992, p. 368). Reproduced with the kind permission of Elsevier Ltd

Table 1.4 Critical packing parameters predict mesophase structures

Molecular structure	Packing parameter	Shape	Mesophase
Small single-tail lipid Large polar head group	<1/3	Cone	Micelle
Single-tai lipid Small polar head group	1/3–1/2	Truncated cone	Hexagonal
Double-tail lipid Large polar head group	1/2–1	Truncated cone	Vesicle
Double-tail lipid Small polar head group	−1	Cylinder	Lamellar
Double-tail lipid Small polar head group	<−1	Inverted truncated cone	Inverted Micelle

Adapted from Israelachvili (1992, p. 381)

1.4 Functionality of Food Emulsifiers

In addition to their primary functions of forming and stabilizing emulsions, dispersions, and foams, food emulsifiers can provide other functionalities. For example, sodium stearoyl lactylate can form a complex with starch, preventing retrogradation (staling) in bread. Soy lecithin is added to chocolate to reduce its viscosity. Separation of peanut oil from peanut butter may be avoided by addition of a small amount of monoglycerides. The emulsifier forms a network of fine crystals, which immobilize the oil. Table 1.5 describes some specialized functions.

It has been common practice to use blends of emulsifiers to achieve optimum organoleptic properties and stability. Some emulsifiers exhibit synergistic activity, that is, they work better together. One example is the combination of Sorbitan monostearate and Polysorbate 60. In some cases, products require multiple functionalities to be acceptable to consumers. For example, cakes must aerate to produce stable foam, to maintain moisture for good mouth feel, and have a fine even grain for appearance. In the current health-conscious environment, the utility of emulsifier combinations must be balanced against pursuit of a "clean label." Formulators are attempting to replace the most chemical-sounding names with natural food ingredients, wherever possible.

When developing a new product, information sources can save time. For existing products, literature from emulsifier suppliers can be a good starting point. However, as food emulsifiers have become commodities, suppliers have lower R&D budgets and need to rationalize their customer assistance. Small companies may have difficulty getting research assistance. For new-to-the-world products, the product developer and marketer need to define the critical properties that make the product acceptable to consumers. If emulsifiers may provide a benefit, food

Table 1.5 Functionalities of emulsifiers in some foods

Functionality	Surfactant	Food example
Foam aeration/ stabilization	Propylene glycol esters	Cake, whipped toppings
Dispersion stabilization	Mono/diglycerides	Peanut butter
Dough strengthening	DATEM	Bread, rolls
Starch complexation (anti-staling)	SSL,CSL	Bread, other baked goods
Clouding (weighting)	Polyglycerol esters, SAIB	Citrus beverage
Crystal inhibition	Polyglycerol esters oxystearin	Salad oils
Antisticking	Lecithin	Candies, grill shortenings
Viscosity modification	Lecithin	Chocolate
Controlled fat agglomeration	Polysorbate 80, polyglycerol, esters	Ice cream, whipped toppings
Freeze-thaw stabilization	SSL, Polysorbate 60	Whipped toppings, coffee whiteners
Gloss enhancement	Sorbitan monostearate, Polyglycerol esters	Confectionery coatings, canned and moist pet foods

regulations should be examined to see which additives would be permitted. A few rough experiments will determine which emulsifiers work.

Statistical experimental designs are very useful when optimizing ingredient composition. A full factorial design should be carried out first. This design can identify two- and three-factor interactions, which are not uncommon in multiphase products. Once the interactions are identified, smaller fractional factorial designs and response surface methodology (RSM) will be useful to optimize the composition. For products that are prepared by the consumer, robust design can result in products that are less sensitive to small measurement errors.

Although food emulsifiers have become commodities, modern technical tools will continue to generate a deeper understanding of how the additives act to perform their function in complex food systems. The objective of this book is to prepare food professionals and other interested parties to understand how food emulsifiers are made, characterized, and formulated into finished foods and beverages.

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Chapter 2
Synthesis and Commercial Preparation of Food Emulsifiers

Gerard L. Hasenhuettl

2.1 Functional Group Design Principles

Emulsifiers (also called surfactants) are amphiphilic molecules, which contain one or more nonpolar (lipophilic), and one or more polar (hydrophilic) functional groups. For food surfactants, lipophilic groups are straight-chain saturated and unsaturated fatty acids, derived from edible fats and oils. Surfactants for non-food applications are often derived from petroleum, and contain branched and cyclic carbon chains.

Hydrophilic polar groups contain electronegative atoms, such as oxygen, nitrogen, and phosphorous. The nature and number of these polar groups determine whether a surfactant is anionic, cationic, amphoteric, or nonionic, as shown in Fig. 2.1. In anionic surfactants, the negatively charged atom is covalently bonded to the large surfactant molecule, and electrostatically associated with a positive counterion. In cationic surfactants, a positively charged functional group is covalently bonded to the large surfactant molecule, and electrostatically bonded to the negative anion. Amphoteric surfactants have both positive and negative functional groups covalently bound to the lipophilic group. Characteristics of charged surfactants are influenced by the pH of the food system. Nonionic surfactants carry no net charge, but have a dipolar carbon-oxygen bond.

Some food products contain naturally occurring surfactants for example, casein protein, and egg components. Lecithin and lipoproteins are excellent emulsifiers. Leucine, isoleucine, and phenylalanine are non-polar amino acids. Arginine, lysine, and tryptophan are amino acids that contain nitrogen atoms that can impart cationic character to a protein at low pH. Aspartic and glutamic acids

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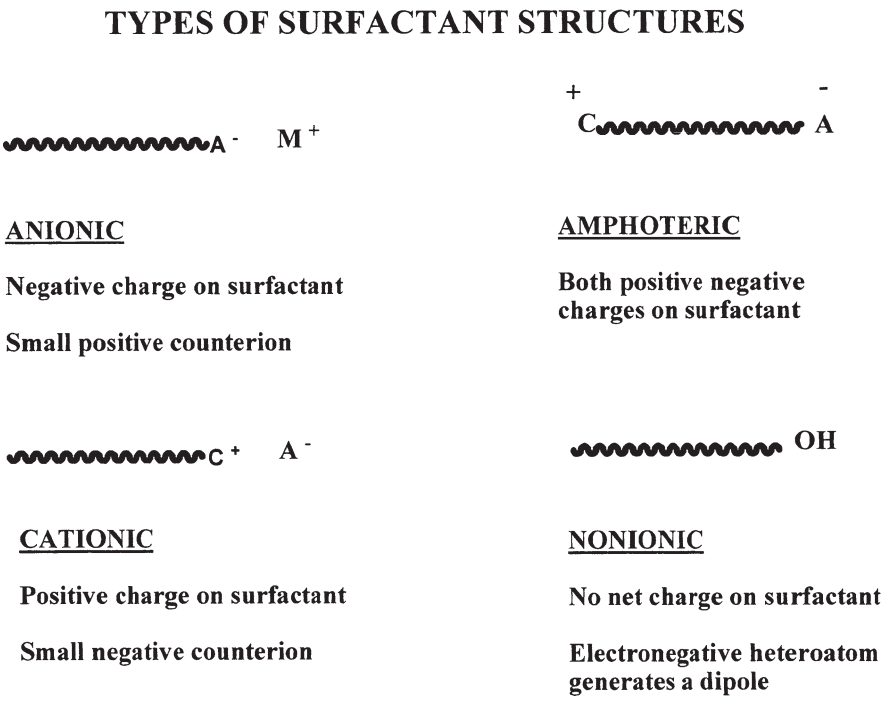

Fig. 2.1 Structures of ionic surfactants

contain carboxyl groups, which contribute anionic character. The nature, number, and location of these charged amino acids determine the isoelectric point of the protein. (The pH at which the protein has a zero net charge) makes the overall molecule amphoteric. Above the isoelectric point, the net charge is negative (anionic). At pH levels below the isoelectric point, the net charge is positive (cationic).

The use of charged surfactants is complicated by their interaction with other charged species, such as calcium and some gums. Proteins may be denatured by high temperatures and shear forces. Egg and soy phospholipids have found many applications in food products. Structurally, these molecules consist of two fatty acids esterified at the 1 and 2 positions, and a phosphates group esterified at the 3-position of the glycerol molecule. Phosphatidyl choline (PC), Phosphatidyl ethanolamine (PE), Phosphatidyl inositol (PI), and Phosphatidyl serine (PS) are the predominant polar functional groups.

Egg and soy lecithin have significantly different structures. They have different PC, PE, PI, and PS distributions. Fatty acids in egg are predominately saturated, while those in soy are mostly unsaturated. This is not surprising, since egg phospholipids are from an animal source, while those from soy are from a vegetable source.

2.2 Chemical Preparation and Modification

A number of synthetic emulsifiers have been used without documented harmful effects. Their chemistry has been developed over a 150 year period (Polouae and Gelis 1844). Early efforts used chemical reactions with fats and oils to produce emulsifiers that were naturally occurring (e.g. mono/diglycerides). Researchers then began to assemble surfactants using building blocks that were used in foods (e.g. acetylated and lactylated monoglycerides). The effort to enlarge the polar head group was accomplished by attaching polyethers to fatty acids (e.g. polyglycerol esters). The fatty acid is cleaved and absorbed in the digestive system, while the polyether passes through the body unchanged.

The lipophilic component of a food surfactant is a fatty acid, obtained from an edible fat or oil. Saturated fatty acids have straight aliphatic chains of 16–22 carbon atoms and no double bonds. Chains of less than 14 carbon atoms, although they are excellent surfactants, contain small quantities of free fatty acids. They impart soapy or goaty off-flavors to finished food products.

Unsaturated fatty acids contain 18–22 carbon atoms and one or two double bonds. More double bonds make the molecule much more susceptible to oxidation. Beany, grassy, and fishy off-flavors are generated by oxidative rancidity. Selective hydrogenation of oils can produce starting fatty acids that are monounsaturated, but also produce trans fatty acids. The trans isomers are undesirable because of their adverse effects on HDL and LDL cholesterol; this can be avoided by using oil that is naturally high in oleic acid, making hydrogenation unnecessary.

2.2.1 Mono/Diacylglycerols

Mono/diacylglycerols, commonly known as mono/diglycerides are the most widely used synthetic surfactants in the food industry. They occur naturally at low levels in fats and oils, due to hydrolysis, an equal concentration of fatty acids are released. Monoglycerides have two free hydroxyl groups, and are more surface-active than diglycerides. There are several chemical reactions that can be used to produce mono/diglycerides in the laboratory. Some involve toxic chemicals, such as acid chlorides and pyridine, that are incompatible with food.

The two most commonly used commercial routes are: (1) Direct esterification of glycerol with a fatty acid and (2) Glycerolysis of a fat or oils shown in Fig. 2.2. Both processes yield a distribution of mono-, di-, and triacylglycerols, free fatty acids, and glycerol. The glycerolysis is more economical, because fats are cheaper than fatty acids, and less glycerol is required. Fats and fatty acids are insoluble in glycerol, and in the absence of solvent, higher temperatures are required to achieve homogeneity.

Direct esterification may be catalyzed either by acids or bases. The ratio of glycerol to fatty acid determines the distribution of mono-, di-, and triacylglycerol's.

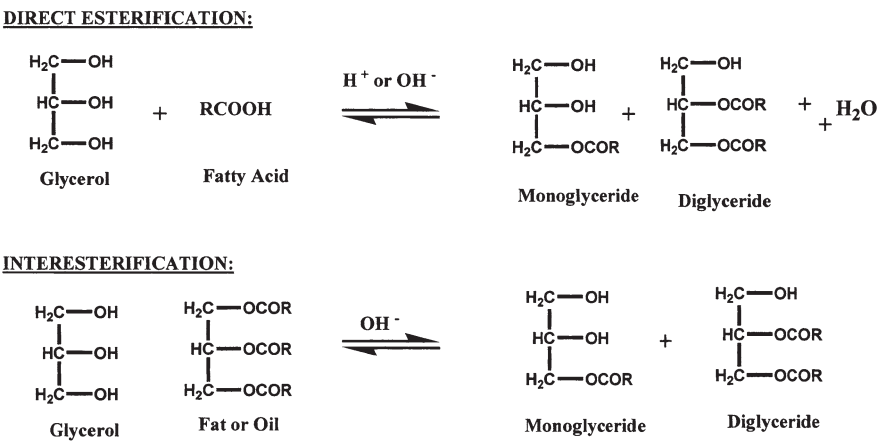

Fig. 2.2 Direct esterification and interesterification

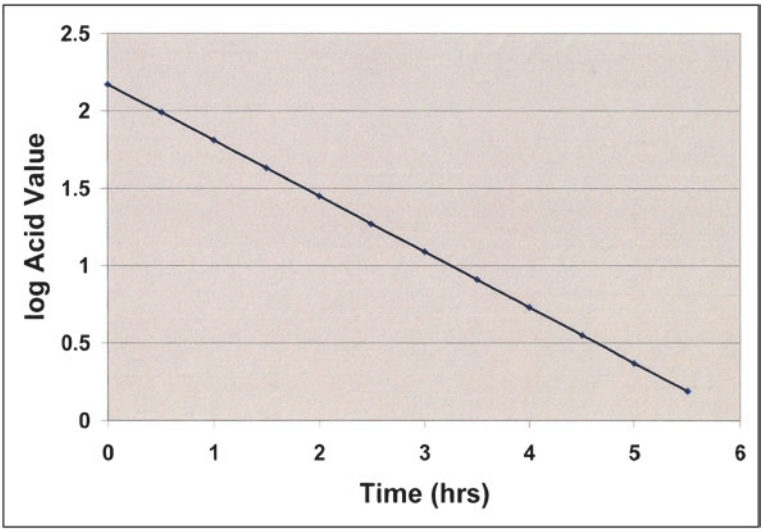

Fig. 2.3 Kinetic plot for direct esterification

Higher glycerol concentrations favor higher levels of monoacylglycerols and free glycerol in the final product. The equilibrium is shifted by continuously removing the water by distillation. Progress of the reaction may be followed by measurement of the acid value (see Chap. 3), or some instrumental method. Figure 2.3 shows the decrease of the log of the acid value versus time. When the reaction is complete, the catalyst is neutralized to stop equilibration. Excess glycerol is then removed by distillation at reduced pressure.

Glycerolysis, also known as interesterification, is accomplished by mixing fat, glycerol, and alkaline catalyst and heating to reaction mixture to high temperatures. Higher glycerol concentrations produce higher yields of monocylglycerols, but also require higher reaction temperatures. The reaction is complete when the reaction is transparent to light. A process has been described in which partial glycerol esters are added to promote homogeneity (Sigfried and Weidner 2005). As with direct esterification, the catalyst is neutralized and excess glycerol is removed. The neutralization step is more critical in a batch process. In a continuous process, glycerol can be flashed off before the mixture can re-equilibrate.

Since these processes are carried out at high temperature, side reactions can occur, which produce dark colors and off-flavors. This can be problematic in many food products. Use of an inert atmosphere, such as nitrogen, can minimize oxidative side reactions. Calcium hydroxide, used as a catalyst at a level of 0.01–0.035%, can produce products with good color and flavor. However, calcium phosphate forms a fine precipitate, which may be difficult to remove with older filters. Low-iron sodium hydroxide (e.g. rayon grade) can also yield products with good quality.

Wee and co-workers (2013) reported a direct esterification method which used hierarchical metal-organic frameworks (MOFS) as heterogeneous catalysts. The process yields monoacylglycerols as the exclusive product. This impressive selectivity is probably due to the small pore of the catalyst. Another advantage to a heterogeneous catalyst is that it can be removed by filtration and re-used. One issue with the cited example is the leaching of small amounts of zinc into the product.

Research using enzymatic reactions to produce emulsifiers, shows that these processes operate at lower temperatures, and can reduce side reactions. Enzymatic reactions will be discussed later in this chapter.

Mono/diglycerides may be produced at concentrations up to 60% by varying the fat/glycerol ratio. The monoglyceride levels can be increased to 90+% by short-path distillation. Mono/diglycerides may be liquid, solid, or semi-solid (also referred to as "plastic"). Liquids may be delivered in pails, drums, tank trucks, or rail cars. Solids may be flaked or beaded. Plastic emulsifiers may be packed into plastic-lined cartons.

2.2.2 Propylene Glycol Esters

Propylene glycol and glycerol have closely related chemical structures. Propylene glycol has one less hydroxyl group, which makes it less polar, lower boiling, and more oil soluble. These differences mean that a lower reaction temperature is required for esterification and transesterification. Figure 2.4 shows direct esterification and interesterification. The processes are similar to those for glycerol, but the distributions are different for the two processes. In addition to propylene glycol mono- and diesters, interesterification also produces mono-, di-, triglycerides, and free glycerol. Some differences in functionality may exist between the two products. Like monoglycerides, the interesterification route is more economical.

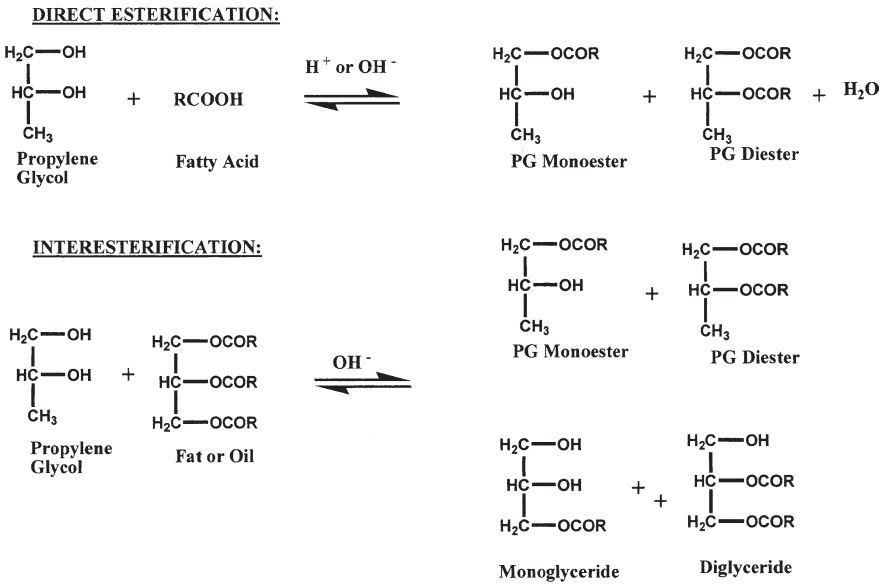

Fig. 2.4 Direct esterification and interesterification to produce propylene glycol esters

Direct esterification of propylene glycol with fatty acids can be catalyzed by acids or bases. As with esterification of glycerol, progress of the reaction is monitored by the decrease in acid value, or an appropriate physical method. After completion, the catalyst is neutralized, and the excess propylene glycol is removed by distillation at reduced pressure. Although fatty acids are expensive, this process may be used where flavor, color, or specific functionality are critical in a finished food product. Propylene glycol monoesters may be separated from diesters by distillation at reduced pressure.

As with glycerolysis, fat, propylene glycol, and an alkaline catalyst are heated in an inert atmosphere. In this process, it is critical to ensure the absence of water, since it retards the reaction. As with monoglycerides, homogeneity (transparency) indicates that the reaction is complete. The concentration of propylene glycol monoesters may be controlled by the ratio of fat to propylene glycol, and measured by gas-liquid chromatography (see Chap. 3).

2.2.3 Polyglycerol Esters of Fatty Acids

Oligomerization of glycerol, followed by esterification with fatty acid, allows the designer to enlarge the size of the polar head group. The hydrophile-lipophile balance (HLB) and mean molecular weight are determined by the degree of polymerization, and the degree of esterification with fatty acid. These factors, along with the

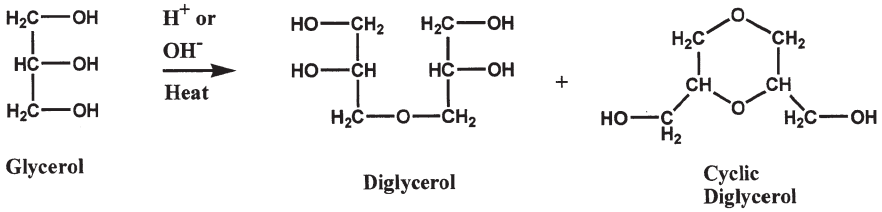

Fig. 2.5 Polymerization of glycerol

nature of the fatty acid, determine whether the product is liquid solid, or semi-solid.

In the first step of this synthesis, shown in Fig. 2.5, glycerol is heated to a high temperature in the presence of an acid or base catalyst under an inert atmosphere. Free hydroxyl groups condense to split out water and form ether linkages. Condensation may be intermolecular to produce linear oligomers, or intramolecular to produce cyclic species. Lower pH and lower temperatures favor the formation of cyclic isomers. When sodium hydroxide is used as the catalyst, the pH declines, as the reaction progresses. Side reactions occur at high temperatures to produce dark colors, off-flavors, and objectionable odors. Processes have been developed using mesoporous (Charles et al. 2003) and zeolite (Esbuis et al. 1984) catalysts under milder conditions. Progress of the reaction may be monitored by refractive index, infrared spectroscopy, or hydroxyl value (see Chap. 3) The viscosity of the polyglycerol increases as the degree of polymerization increases. The degree of polymerization for polyglycerol in the food industry, range from 2 to 12 glycerol units. Polyol distribution can be measured by converting the free hydroxyl groups to trimethylsilyl ethers, followed by gas-liquid chromatography (Sahasrabuddhe and Chadha 1969; Schuetze 1977). Polyglycerol may be used as produced, or stripped of excess glycerol by distillation at reduced pressure (Aoi 1995).

Direct esterification with fatty acids or interesterification with fats or oils can be used to produce polyglycerol esters. For polyglycerols with higher degrees of polymerization, fatty acids are used, to avoid introducing glycerol into the product. Interesterification may be used for polyglycerols, which have been stripped of free glycerol and cyclic diglycerol. The HLB of the surfactant is controlled by the molar ratio of fat (or fatty acid) to polyglycerol. High reaction temperatures cause undesirable side reactions. A lower temperature process, which uses a solid catalyst, has been described (Marquez-Alvarez et al. 2004).

A unique surfactant is produced by the reaction of polyglycerol with bifunctional ricinoleic acid, the predominant fatty acid in castor oil. The carboxyl group of ricinoleic acid may react with a hydroxyl group on the polyglycerol, or with a hydroxyl group of another molecule of ricinoleic acid. The composition of the reaction mixture can be controlled by the order of addition (Aoi 1995).

2.2.4 *Sorbitan Monostearate and Tristearate*

Despite its simple name, sorbitan monostearate is a complex mixture of molecules. Commercial stearic acid may have a range of 45–90% C18:0, depending on its source. Esterification and dehydration/cyclization reactions occur simultaneously. Cyclization produces a mixture of sorbitol, sorbitan, and isosorbide. Esterification yields a distribution of mono- through hexastearate. Sorbitan monostearate and tristearate are averages of their respective distributions. This is controlled by the fatty acid/sorbitol ratio.

A reaction mixture of stearic acid, sorbitol, and an alkaline catalyst in an inert atmosphere, Fig. 2.6 shows the simultaneous cyclization and esterification reactions. Water is continuously removed by distillation. Sodium hydroxide (Griffin 1945) and zinc stearate (Szabo et al. 1977) have been used as catalysts. Because of the high temperatures required to achieve homogeneity, caramelization side reactions occur, which produce dark colored materials. These side reactions may be reduced by inclusion of a reducing agent, such as sodium hypophosphate (Furuya et al. 1992). An alternative approach uses an acid catalyst to carry out the cyclization reaction at a lower temperature (Stockburger 1981). The polyol mixture is purified and reacted with stearic acid to produce the emulsifier. As with monoacylglycerols, progress of the reaction may be monitored by measurement of the acid value. Infrared spectroscopy can be used to measure disappearance of the free hydroxyl group. Although these methods are fairly rapid, they do not provide any information about the molecular distribution. Gas-liquid chromatography has been shown to provide this information (Sahasrabuddhe and Chadha 1969; Franzke and Knoll 1980).The reaction mixture may also be measured by HPLC (Garti and Asarin 1983). Unfortunately, these instrumental methods are time consuming. The final product must meet tight FDA specifications for saponification and hydroxyl values (see Chap. 3). Sorbitan monostearate and monooleate are used as intermediates in the production of polysorbates, discussed later in this chapter.

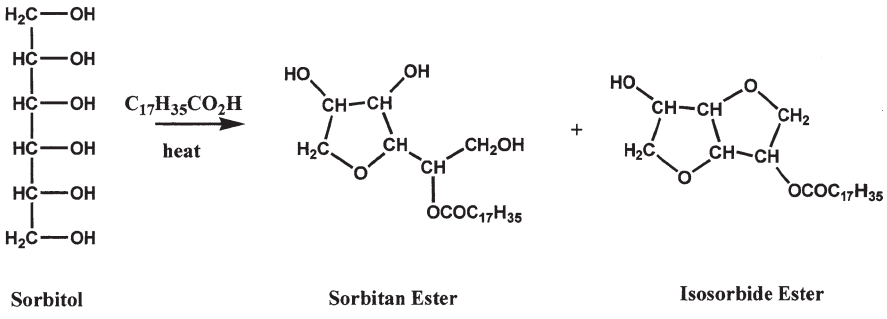

Fig. 2.6 Preparation of sorbitol and isosorbide esters

2.2.5 *Sucrose Esters*

Sucrose polyesters have been widely investigated as zero-calorie replacements for fats in food (Akoh and Swanson 1994; Swanson and Swanson 1999). These ingredients are fully esterified sucrose fatty acid esters, and have properties similar to fats and oils. Partially esterified sucrose esters are versatile food emulsifiers. A typical reaction scheme is shown in Fig. 2.7. The distribution of mono-, di-, and triesters, and therefore the HLB, are controlled by the fatty acid methyl ester (FAME)/sucrose ratio in the reaction mixture. The saturation and chain length of the fatty acids also influence physical and functional properties.

Like other polyols, sucrose has low solubility in fats and fatty acids. However, since sucrose undergoes caramelization above 140 °C., high temperatures cannot be used to force homogeneity. One strategy is to carry out a base-catalyzed interesterification in a polar organic solvent, such as dimethylformamide (Wagner et al. 1990) or dimethylsulfoxide (Kasori and Taktabagai 1997). The disadvantage of a solvent based method is the difficulty of complete removal of the toxic low volatility solvent.

Another approach is the use of high levels of soap, or other surfactants, to promote miscibility of the phases (Murakama et al. 1989). Fittermann et al. (2012) has suggested that co-melting of sucrose with a soap of a multivalent cation produces a homogeneous reaction mixture (Zhao et al. 2014). The excess soap may be removed by neutralization to the fatty acid, followed by short path distillation. Alternatively, a volatile solvent, such as ethyl acetate blended with water, can separate the mixture by extraction. Sucrose octaacetate, an oil soluble material, may be reacted with methyl esters in a homogeneous reaction mixture (Elsner et al. 1989). A continuous process, in which a heated reaction mixture is passed through an immobilized catalyst, has been described (Wilson 1999). A mixture of sucrose ester and mono/diglycerides can be produced by interesterification of fats and sucrose (Nakamura et al. 1986).

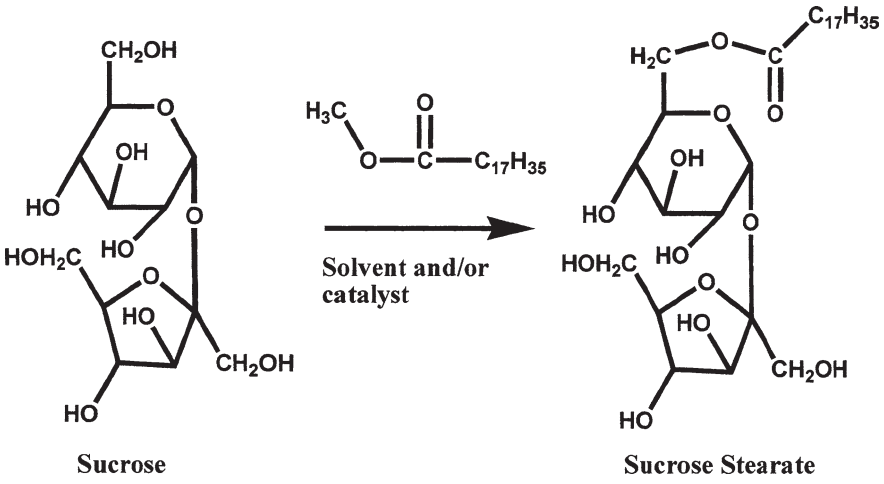

Fig. 2.7 Preparation of sucrose ester surfactants

Reaction of two moles of acetic acid and six moles of isobutyric acid with one mole of sucrose produce an emulsifier with high specific gravity. The resulting additive, sucrose acetate isobutyrate (SAIB), is used as a weighting agent to disperse oils in beverages (Reynold and Chappel 1998).

Composition of reaction products may be obtained by thin layer chromatography (TLC) (Li et al. 2003), or by reverse phase high-performance liquid chromatography (RPHPLC) (Murakama et al. 1989; Okumura et al. 2001). Degree of esterification can also be determined by electrospray mass spectrometry (Schuyl and Platerink 1994).

2.2.6 *Sodium and Calcium Stearoyl Lactylate*

A surfactant with a carboxylic acid group may be nonionic, or reacted with sodium or calcium hydroxide to form an anionic molecule. Lactic acid is a bifunctional molecule that can self-condense to form an oligomer, or react with stearic acid to give stearoyl lactylic acid (Eng 1972). Figure 2.8 shows the dimeric homolog, commonly known as sodium stearoyl lactylate. In a typical preparation, lactic acid is neutralized with sodium hydroxide, and the excess water is removed by distillation. Iron is highly detrimental to the quality of the product. Consequently, raw materials should contain minimal iron, and the reactor should not contribute leachable iron. Stearic acid is added and esterification is carried out at 160–180 °C. Higher temperatures lead to side reactions, which give rise to off flavors and disagreeable odors and flavors. Water is continuously removed by distillation, and acid value is monitored until the desired value is reached.

The color of the product may be improved by bleaching with 30% hydrogen peroxide, followed by heat treatment to destroy the excess peroxide (Anon. 1983). The final product is characterized by acid value, saponification number, and total lactic acid (Franzke and Knoll 1980).

2.2.7 *Derivatives of Mono/Diacylglycerols (Mono/Diglycerides)*

Mono and diacylglycerols have a significant mass of lipophilic functionality. The hydroxyl head group is small and nonionic. The size and charge of the head group may be modified by attaching another polar functional group. The result is an increase in the hydrophilicity of the surfactant.

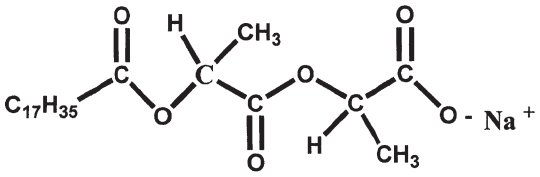

Fig. 2.8 Structure of sodium stearoyl lactylate

2.2.7.1 Acetylated Monoacylglycerols

Reaction of an acetic acid derivative results in replacement of a free hydroxyl group with an acetate group. This change produces a less hydrophilic surfactant, which structurally resembles a fat. Because of their diversity in alkyl chain length, acetylated monoacylglycerols are excellent film formers (Guillard et al. 2004).

Two methods are commonly used for the preparation of acetylated acylmonoglycerols: (1) Monoacylglycerols are reacted with acetic anhydride to produce the acetate ester and one equivalent of acetic acid. The reaction is catalyzed by a strong mineral or organic acid. If the reactor is suitably equipped, the acetic acid may be removed by distillation, and recycled to produce acetic anhydride. (2) Monoacylglycerols can be reacted with glycerol triacetate (triacetin) using an alkaline catalyst. Although acetic acid is not produced as a byproduct of the reaction, glycerol and its mono-, di-, and triacetate esters must be removed by distillation. The advantage of this process is that the reactants and products are less flammable and corrosive.

2.2.7.2 Lactylated Monoacylglycerols

As previously discussed, lactic acid is a bifunctional molecule. Reaction of its carboxyl group with a monoacylglycerol, a lactylated monoacylglycerol is formed. The hydrophilic head group is enlarged, but the nonionic character is maintained. Synthesis is accomplished by heating a monoacylglycerol with lactic acid. The temperature must be kept below 180 °C. to avoid caramelization reactions. Kinetics of the reaction are similar to the direct esterification of glycerol with fatty acids. Water is continuously removed by distillation. The degree of esterification is controlled by the ratio of monoacylglycerols to lactic acid (Shmidt et al. 1976). After the reaction is complete, lactate esters of free glycerol must be removed because they impart off-flavors to the finished food product. Steam distillation or aqueous extraction are useful techniques to accomplish the task. The product may be characterized by acid value, saponification number, water insoluble combined lactic acid (WICLA), and chromatography.

2.2.7.3 Succinylated Monoacylglycerols

Succinic anhydride is similar to acetic anhydride in its esterification of free hydroxyll groups. However, since an alkyl group tethers the two carboxyl groups, the second carboxyl group is retained in the surfactant molecule, rather than expelled as an acid by product. The polar head group is enlarged and can become anionic at the appropriate pH.

In a typical synthesis, a purified monoacylglycerol is reacted with succinic anhydride under an inert atmosphere (Freund 1968; Hadeball et al. 1986). Precautions must be taken while handling succinic anhydride, a suspect carcinogen (Sax and Lewis 1999). Although the reaction is exothermic, heat is initially added to raise the temperature to 150–165 °C. to achieve homogeneity of the mixture. Since succinic acid is bifunctional, it may bind to two monoacylglycerol molecules.

2.2.7.4 Monoacylglycerols Citrate (CITREM)

Citric acid has multiple carboxyl groups, one of which can condense with monoacylglycerols. This increases the size and polarity of the head group. In addition to its surface activity, citrate esters can chelate metals, such as iron and copper, which catalyze oxidation.

Preparation of monoglycerides citrate is carried out by reacting monoacylglycerols with citric acid or its anhydride in the presence of an acid catalyst (Bade 1978). The anhydride method can be performed at a lower temperature. However, this process is more expensive because of the extra step needed to produce the citric anhydride. When citric acid is used, temperatures above 130 °C. must be avoided to prevent decomposition.

2.2.7.5 Diacetyltartaric Esters of Monoacylglycerols (DATEM)

DATEM surfactants are formed from the condensation of a monoacylglycerol with diacetyltartaric acid. In this case, the acetyl groups block the self-condensation of tartaric acid. The head group is enlarged, and can become anionic above its pK_a value.

Synthesis is accomplished by either a two or three step process: (1) Tartaric acid is reacted with acetic anhydride, using sulfuric acid as a catalyst, to produce diacetyltartaric acid. (Gladstone 1960); (2) Optionally, diacetyltartaric acid may be converted to its anhydride; (3) Diacetyltartaric acid, or its anhydride, is reacted with a monoacylglycerol. As with CITREM, the anhydride method uses a lower reaction temperature, but is more expensive.

2.2.7.6 Monoacylglycerol Phosphate

Attachment of a phosphate group to a monoacylglycerol incorporates a polar group with larger size, and the potential to be anionic. The synthesis comprises reaction of a monoacylglycerol with phosphoric acid (Cawley and Grad 1969), polyphosphoric acid (Kazyulima et al. 1986), or phosphorous pentoxide. As with other reactions described in this chapter, the reaction mixtures are initially heterogeneous, but as the reaction progresses, the surfactant product coalesces into a single phase. Alternatively, a solvent may be used to carry out the reaction at a lower temperature. A synthesis directly from triacylglycerol has been reported (Ranny et al. 1989).

2.2.8 Polyoxyethylene Derivatives

Ethylene oxide (oxirane) is a molecule with a three membered oxygen containing ring. Since the ring strain is high, the molecule can readily undergo an exothermic SN2 ring opening reaction. The open ring nucleophile can react with another oxirane molecule to propagate a polymerization chain reaction.

Surfactants have been synthesized by using fatty acids or fatty alcohols as initiating nucleophiles. The product has a very large nonionic head group, which can also chelate cations to a small extent. In the food industry, sorbitan esters and monoacylglycerols have been ethoxylated to form higher HLB surfactants.

2.2.8.1 Polyoxyethylene Sorbitan Ester (Polysorbates)

The preparation of sorbitan esters was previously discussed. Although sorbitan monooleate is not approved for food use in the United States, its ethoxylated derivative is listed. The common nomenclature of sorbitan esters and polysorbates has evolved from the trade names of products marketed by ICI Corporation.

A number of challenges arise in the synthesis of ethoxylates. Oxirane has a boiling point of 10.4 °C. (Udajari 1996), making it a gas at ambient temperature. It is also a suspect carcinogen, so it must be tightly contained to avoid exposure. Ethylene oxide may also dimerize to dioxane, another suspected carcinogen, during the reaction. Care must therefore be taken to remove it from the product.

Unlike other reactions in this chapter, ethoxylation is exothermic. Efficient mixing and heat exchange are necessary to avoid an explosion. In a typical preparation (Fig. 2.9), a sorbitan ester is introduced into a pressure reactor, similar to that used for hydrogenation. Ethylene oxide is slowly added while cooling to remove the heat of reaction. After the reaction has been completed, the product is purified by steam distillation in order to remove any traces of dioxane.

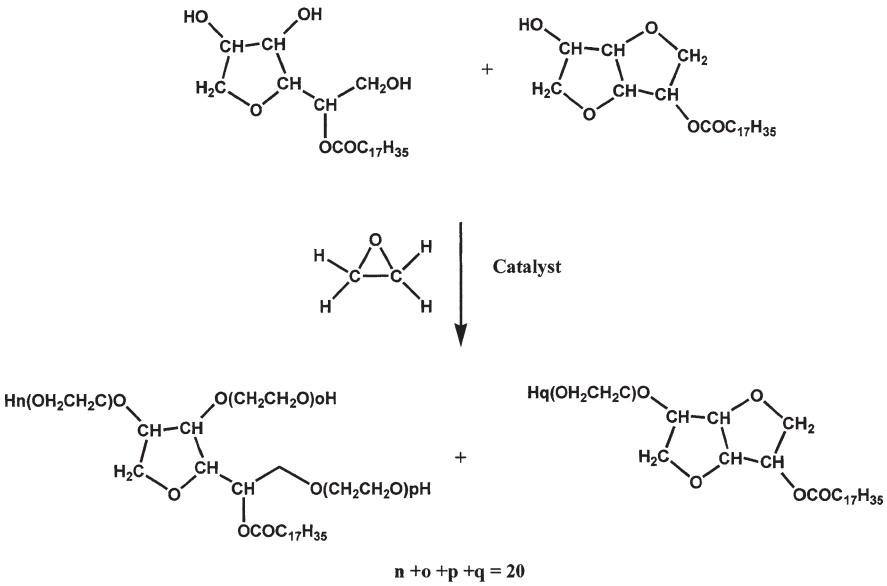

Fig. 2.9 Preparation of polysorbate surfactants

Saponification number, hydroxyl value, and polyoxyethylene content are used to characterize the surfactant. Negative ion mass spectrometry has been used to determine the distribution of polyoxyethylene chains (Brumley 1985). This technique can be a valuable tool to sort out subtle structure function relationships.

2.2.8.2 Ethoxylated Monoacylglycerols

Preparation of this surfactant is carried out in two stages: (1) Preparation of the monoacylglycerols from fats or fatty acids is done as previously described. However, in this case, the alkaline catalyst is not neutralized, but carried over to the second reaction. (2) Ethoxylation is carried out in a fashion similar to polysorbates, but the temperature is raised to 170–180 °C., in order to initiate the reaction. The product is steam or nitrogen deodorized to remove traces of dioxane. Excess catalyst is removed by filtration.

2.2.9 Chemical Modification of Naturally Occurring Molecules

Many naturally occurring materials have been used to impart textural characteristics to food products. Some of these properties may be improved by chemical modification of the hydrophilic head or lipophilic tail. The result is a hybrid molecule; which is partly natural and partly synthetic. Other approaches are carrying out the reaction with an enzyme, or fractionating the natural material with a solvent. In these cases, it is less clear whether the modified products are "natural."

2.2.9.1 Modified Lecithin

Lecithins are found in plants and animals as essential components of membranes. Soy lecithins are widely used in the food industry (Szuhaj 2005). The crude product is inexpensive, because it is a by-product of refined soybean oil. Egg lecithin, derived by extraction of yolk, is a very functional emulsifier. However, it is more expensive and less stable than soy lecithin. Structurally, the major feature is a glycerol backbone, with fatty acids esterified at positions 1 and 2, a phosphate at position 3, and a variety of substituents bonded to the phosphate (see Fig. 2.10).

Soy lecithin is a complex mixture of phospholipids and entrained soy oil. Solvents may be used to separate lecithin from these triacylglycerols, for example, acetone. Egg lecithin may be fractionated into its constituents by sequential extraction with ethanol, hexane, and acetone (Palacios and Wang 2005). In soy lecithin, phosphatidylcholine may be enriched by extraction with ethanol (Gu 2002). A second hydrophilic group may be introduced into soy lecithin by hydroxylation with hydrogen peroxide (Sietze 1982). This is a four-centered reaction that adds oxygen across an alkyl chain double bond. Incorporation of the remote polar group allows the molecule to adopt an "inchworm" structure at an oil-water interface.

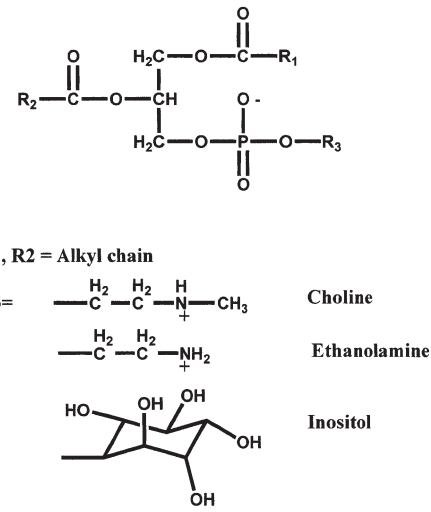

Fig. 2.10 Structure of lecithins

2.2.9.2 Propylene Glycol Alginate

Alginic acid is a polar hydrocolloid polymer, containing hydroxyl and carboxyl groups. It is extracted from seaweed, and is a copolymer of mannuronic and guluronic acids. The sodium and calcium salts form gels, and are used as thickeners in food products. These ingredients have little surface activity.

Esterification with propylene glycol or propylene oxide reduces the hydrophilic character of the molecule. Approximately 80% of the carboxyl groups can be esterified (McDowell 1970, 1975). Figure 2.11 shows the esterified polymer. Propylene oxide is a volatile liquid with a boiling point of 34 °C. Like ethylene oxide, propylene oxide is flammable, and exposure can cause burns and blistering.

In a typical preparation, a concentrated alginic acid is reacted with propylene oxide in a pressure reactor at 65–80 °C for 30–60 min (Nielson et al. 1971). The degree of esterification can be improved by neutralizing the alginic acid with sodium hydroxide (Noto and Petit 1972; Strong 1976; Ha et al. 1987).

2.2.9.3 Modifications of Cellulose

Cellulose is a carbohydrate polymer of glucose, which differs from starch in the stereochemistry of the linkage between monomers. It has a very tight structure, and is used as a source of fiber in food products. It has poor water solubility, but the numerous hydroxyl groups can include water and cause the structure to swell.

Lipophilic groups can also be introduced to provide surface activity. Methyl and ethyl chloride can react to form alkyl ethyl ethers. Chloroacetic acid can react to produce carboxymethyl cellulose. Analogous to alginic acid, cellulose can react with

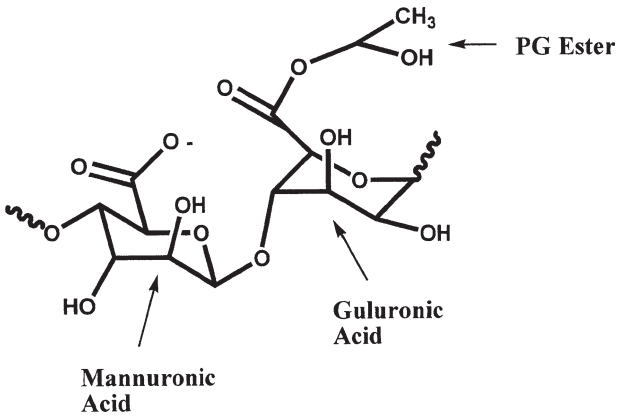

Fig. 2.11 Monomeric unit of propylene glycol alginate

propylene oxide to give hydroxypropyl cellulose. The degree of substitution is determined by the ratio of the reactants, and the reaction conditions (Belitz et al. 2004a).

2.3 Preparation of Naturally Occurring Emulsifiers

There has been a trend among consumers over the last decade to seek out natural foods, and to avoid genetically modified ingredients. Manufacturers, who are trying to meet these demands, still face the problems maintaining texture and phase stability. One strategy is to employ naturally occurring compounds that are surface-active.

2.3.1 Lecithin

Lecithins constitute a group of compounds, mainly based on a glycerol backbone. Fatty acids are esterified at the 1- and 2-positions and a phosphate bonded at the 3-position. Also bonded to the phosphate are mixtures of choline, ethanolamine, serine, and inositol. Sphingosines and glycolipids (non-glycerin based) may also be present. The presence of phosphate and nitrogen allow the emulsifier to be anionic, cationic, or zwittterionic, depending on the pH.

Lecithins are found in plant, marine, and animal membranes. Animal lecithins contain higher levels of saturated fatty acids than vegetable-derived products. The distribution of species bonded to the phosphate group, are also different. Oilseed lecithins contain mostly phosphatidylcholine. Egg lecithin contains a flatter distribution of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine. The two major commercial sources of lecithin for the food industry are soybeans and eggs.

2.3.1.1 Soy Lecithin

Lecithin is a by-product of crude soybean oil. It must be removed because it interferes with downstream bleaching, hydrogenation and deodorization of the oil. This is accomplished by injecting steam and water into the oil, followed by centrifugation or skimming. The process is also known as degumming or desliming. This process may be facilitated by hydrolytic enzymes, which further decreases mutual solubility, and also increases the HLB of the lecithin. An interesting side note is that enzyme-hydrolyzed lecithin is permitted in Europe, but has not yet been formally approved in the United States. Crude lecithin may contain up to 30–40% residual oil.

Lecithin can be further purified. A solvent, such as acetone, can be used to remove residual nonpolar oils. The solid lecithin has improved emulsification properties. Ethanol fractionation can be used to separate phosphatidylinositol and phosphatidic acid from phosphatidylcholine. The purified PC contains a small amount of residual phosphatidylethanolamine.

2.3.1.2 Egg Lecithin

Egg yolk is composed of nonpolar lipids, proteins, and lecithins. The lecithin can be purified by solvent extraction, fractionation, or solid-liquid absorption chromatography. The purified products are used in the pharmaceutical and specialty nutritional industries, but are too expensive for most processed food products. Egg yolks or whole eggs are used in mayonnaise and bakery products. Egg yolks may be dried and powdered to improve microbial stability.

2.3.2 Milk Proteins

Milk is a colloidal dispersion of butterfat, stabilized by a milkfat globule membrane. Casein is the main interfacial active protein responsible for phase stability. Whey is the other commercially important milk protein. It has a hydrophobic pocket that may be useful for carrying lipids and flavors. A nutritional function of milk proteins is the delivery of calcium and phosphorous to the neonate. For a detailed treatment of chemistry, genetics, and processing, Thompson, et al. is recommended (Thompson et al. 2009).

Milk proteins can be separated by precipitation or treatment with enzymes. Casein proteins are precipitated when the pH drops near their isoelectric points. This likely occurs because of reduced electrostatic repulsion of milkfat globules. Belitz (2004a) has shown how charged and hydrophobic amino acid residues are arranged along the casein backbone. Whey protein and lactose remain in the supernatant. This transformation occurs during the process of cheese making, but is also useful to produce protein ingredients.

Whey proteins are by-products of cheese production. Since the price is low, many applications for it have been developed. The proteins are globular in structure, composed mainly of β-lactoglobulin, β-lactalbumen, and immunoglobulins (Belitz et al. 2004b).

2.3.3 Surface-Active Hydrocolloids

Hydrocolloids, also commonly referred to as gums, are widely used to stabilize emulsions and dispersions. Their main mechanism of action is to thicken the continuous aqueous phase, thus reducing the mobility of the dispersed phase. Since these are natural, minimally processed additives, some contain proteins that have surface activity.

2.3.3.1 Gum Arabic

Gum Arabic is an exudate of acacia trees, primarily *Acacia Senegal*. It is produced in Sudan and other African countries. It is obtained by gashing the tree bark and collecting the air-dried exudate. The gum is dissolved in 0.1 molar hydrochloric acid and precipitated with ethanol.

The polymeric structure of the hydrocolloid is L-arabinose, L-rhamnose, D-galactose, and D-gluconic acid. The carboxyl group provides the ionic character. Approximately 2% of a protein is bound to the carbohydrate, and is largely responsible for its interfacial and film forming activity.

2.3.3.2 Guar Gum

Guar (or guaran) gum is obtained from the leguminous plant *Cyanopsis tetragonoloba*. The plant is grown for forage in India, Pakistan, and the United States. In the milling process, the hull and germ are removed from endosperm to produce guar flour.

The carbohydrate structure consists of a linear chain of β-D-mannopyranose units joined by 1–4 linkages. Branches of α-D-galactosylpyranose units are attached at every second chain unit. Guar gum has a protein content of 5–6%, which gives the ingredient some surface activity.

2.3.4 Locust Bean Gum

Locust bean gum, also known as carob and St. John's gum, is obtained from the seed pods of an evergreen, cultivated in the Mediterranean area. The seeds consist of hull material, germ and endosperm. The seeds are milled and the endosperm is removed and utilized as flour, analogous to guar.

The flour is approximately 88% galactomannan and 6% protein. The carbohydrate structure is made up of a linear chain of 1–4 linked β-D-mannopyranose units, with α –D-galactopyranose residues linked as branches at every 4th to 5th unit.

2.4 Commercial Preparation of Food Surfactants

Synthesis in the laboratory and manufacturing on a commercial scale are often different. On a small scale in glass equipment, corrosive and toxic chemicals can be handled, and products can be purified by chromatography. Although glass-lined commercial reactors are available, they are vulnerable to breakage and development of pinhole leaks. Chromatographic purification is expensive, meaning that it is only useful for high-value products.

The choice between batch and continuous process depends on product volume and product mix. A continuous process is best suited to one or a few products, which are produced in large quantities. A large number of products, produced in smaller quantities, are best handled with batch processes. Direct esterification reactions with fatty acids have traditionally been batch processes, due to the slow reaction rates.

2.4.1 Batch Esterification/Interesterification

Commercial batch reactors are usually constructed of carbon or stainless steel. High molybdenum stainless steel is preferred when acids are used in the process. For example, fatty acids at high temperatures are corrosive.

In a typical direct esterification or interesterification process, a fat or fatty acid, polyol and a catalyst are weighed or metered from storage tanks into the reactor. A nitrogen atmosphere is maintained, and heat is supplied from heating coils or a jacket. When the reaction is complete, the catalyst is neutralized, and the reaction mixture is cooled by coils or a jacket. Often, the heating and cooling coils are separate. A high-boiling heat exchange fluid is used to raise the temperature, while water is used for cooling. To conserve energy, hot water exiting the reactor, can be used to pre-heat reactants for the next batch.

Excess polyol is removed by distillation, gravitational separation, or extraction. The product is filtered and pumped to storage.

Figure 2.12 shows a schematic of a typical batch reactor. Some critical design factors for batch reactors are: (1) The reactor, piping, and storage tanks must be constructed of corrosion-resistant materials. In addition to damaged equipment, any iron or copper leached into the product can become a pro-oxidant. (2) Meters and or scales, used to measure reactants, must be sufficiently accurate to meet product specifications. (3) If excess polyols are to be recycled, fractional distillation efficiency must be sufficient to prevent cross contamination of succeeding batches. (4) Sufficient heat capacity must be designed to attain rapid heating and cooling cycles. (5) Starting oil storage, the reactor, filter assembly, and product storage must be

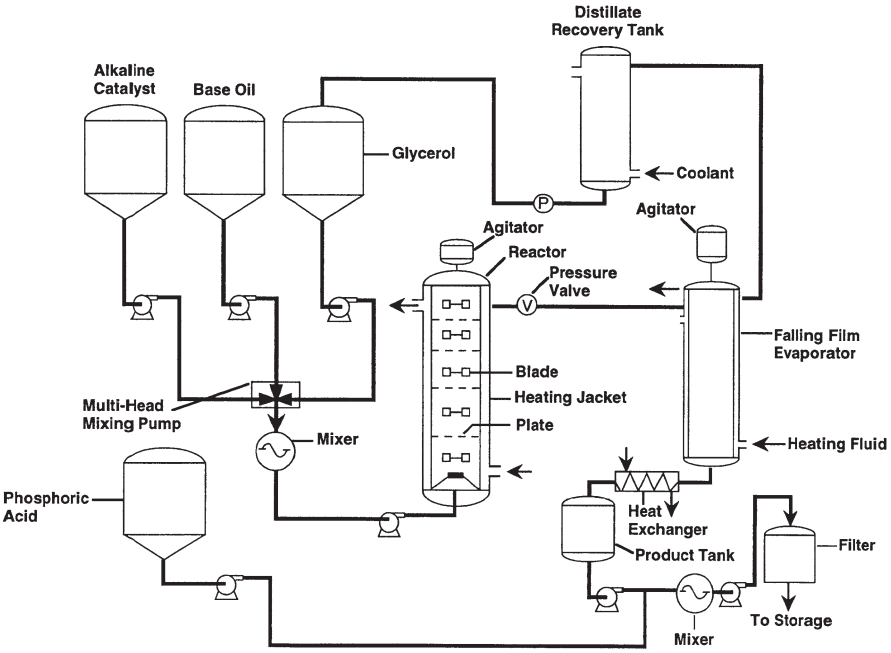

Fig. 2.12 Schematic of a batch esterification reactor

protected by an inert atmosphere, to prevent oxidation. (6) An adequate cleaning system is necessary to avoid cross-contamination between batches. (7) An effective waste treatment system is necessary to minimize environmental impact.

2.4.2 Continuous Esterification Reactors

Continuous processes are generally economical for manufacturing large volumes of product. Once conditions are established, large amounts of products are continually produced, as long as the process remains under control. In a typical continuous reactor, as shown in Fig. 2.13 (Allen and Campbell 1967), oil, catalyst, and polyol are metered into the flow-through reactor through a multiplex pump. At high temperature, homogeneity is rapidly achieved. The reaction mixture is passed through a short path evacuated distillation module, in which excess polyol is removed. Neutralization of the catalyst is not required, as the distillation time is relatively short, compared to the batch process. As a result, the equilibrium does not have sufficient time to shift significantly.

Continuous processes are not well suited to performing direct esterification reactions. The short time in the reaction chamber does not allow water to be removed in sufficient mass to shift the equilibrium. The process would need to be redesigned to a system capable of continuous water removal.

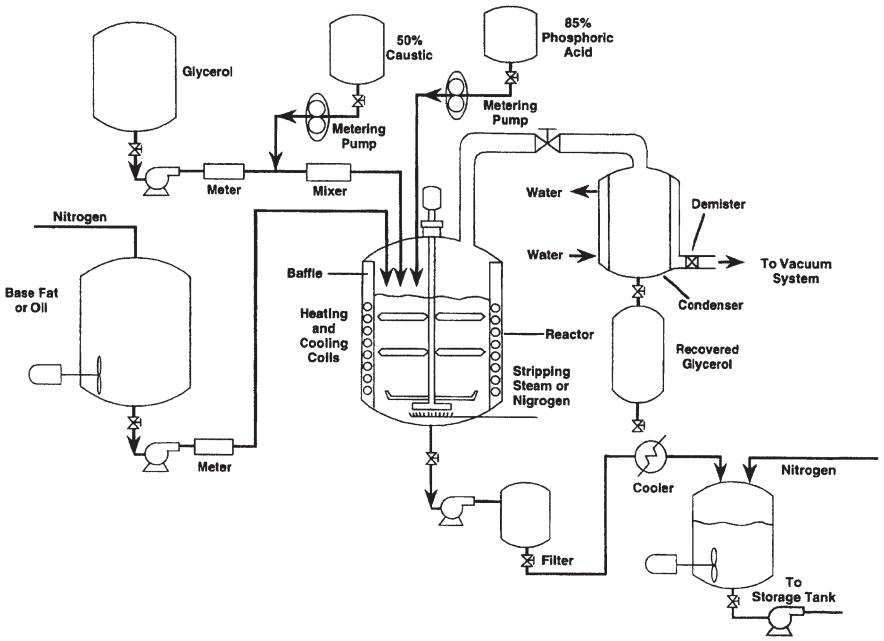

Fig. 2.13 Schematic of a continuous interesterification reactor

The final product is neutralized and filtered. If calcium hydroxide is used as the catalyst, and phosphoric acid is used for neutralization, the filter must be capable of removing the fine precipitate. The clear product may be pumped to storage tanks, or loaded into tank trucks or rail cars.

There are several critical parameters for the design of continuous reactors: (1) the metering pumps must be precise and accurate, so that the process remains stable over time; (2) Materials of construction must not contribute pro-oxidants, such as copper and iron, to the product; (3) Heat transfer to the reactor must be sufficient to maintain a temperature of up to 500 °F. (4) The distillation unit should be sized and configured to rapidly remove polyol, but minimize loss of product due to blow-over.

2.4.3 Enzymatic Reactors as a Transition Technology

Direct and interesterification may be performed using lipase or esterase enzymes instead of chemical catalysts. Enzymatic reactors may also be either batch or continuous. The major advantage of enzymatic processes is that they operate at a lower temperature, and therefore, use less energy. Low temperatures produce fewer side reactions, and may provide improved regioselectivity (for example, esterifying only primary alcohols).

The major disadvantages of enzymatic reactions are the high cost of the enzyme and the slow reaction rate. Slower reaction rates are largely a result of the heterogeneous nature of the reaction mixture.

Enzymatic reactors may be either batch or continuous. As with chemical processes, batch processes are better suited to smaller batches and a wide variety of products. Continuous processes are preferable for large batches and a narrower product line. Stirred tank batch and fixed-bed (continuous) reactors have been used to carry out reactions with enzymes and microorganisms (Patterson et al. 1984; Arcos et al. 2000). Time of exposure to the enzyme and fatty acid/polyol ratio the critical factors controlling the rate and selectivity of the reaction. A flow-through microporous membrane reactor, as shown in Fig. 2.14, has been used to prepare

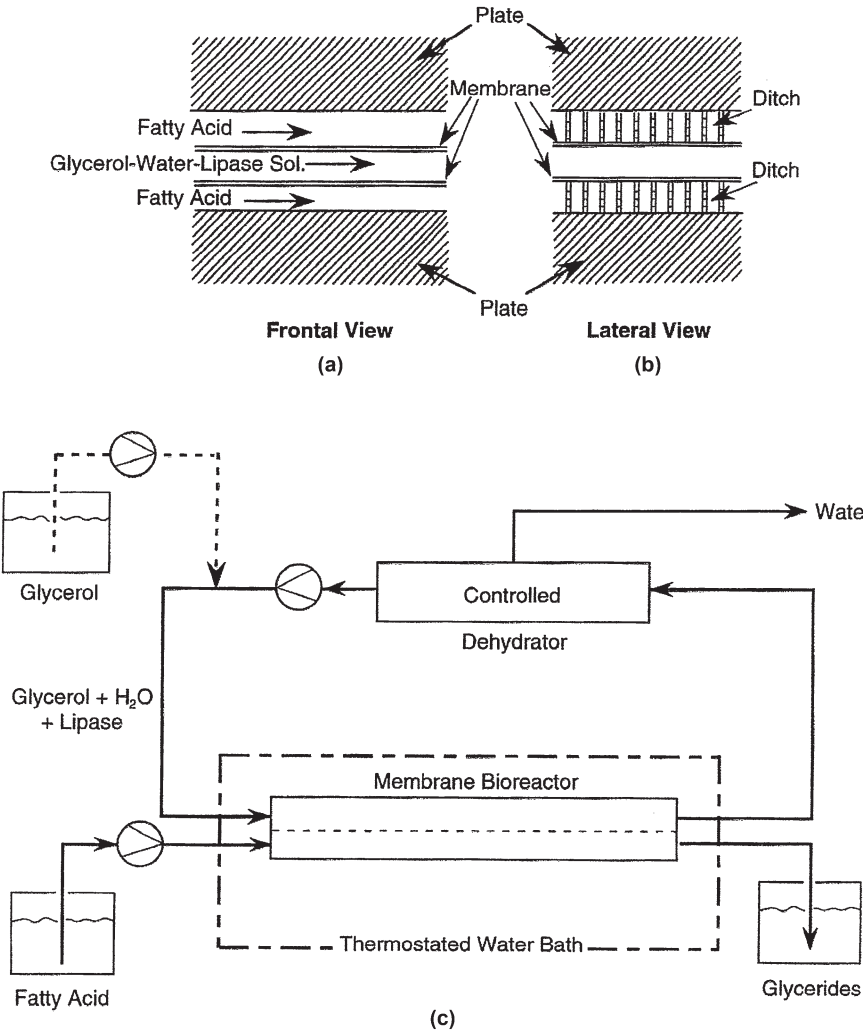

Fig. 2.14 Schematic of an enzymatic membrane reactor

surfactants (Yamane et al. 1984; Hoq et al. 1985). A fatty acid stream is passed along one side of the membrane. Glycerol, lipase, and an activating amount of water are passed along the other side. An evaporative membrane reactor has been developed, and its design parameters reviewed (Lim 2002). Evaporation of water in this reactor shifts the equilibrium toward the products. Deposition of lipid, protein, or chitosan on the membrane improves the contact of the lipid and polyol phases (Paolucci-Jeanean 2005).

2.5 Enzymatic Modification of Natural Food Components

A recent trend among food manufacturers has been the search for a "clean label." However, removal of chemically produced ingredients can present both technical and cost challenges. For example; replacing polysorbate with a natural gum may result in a product with inferior texture, at a higher cost.

One strategy to achieve clean labels and "all natural" claims is to treat naturally occurring products with enzymes that occur in nature. Of course, there will still be debate about genetic modification, but enzymatic reactions are conceptually more natural than high-temperature chemistry.

Even with improvements in heterogeneous catalysis, conventional chemicals require temperatures that cause degradative side reactions. Enzymatic reactions are carried out in living cells at ambient temperatures. In addition, enzymes often have regioselective and enantioselective capability. For food emulsifiers, selectivity has two advantages: (1) Primary alcohols can be esterified in the presence of secondary alcohols (1,3-specific lipase) or vice-versa (2-specific lipase); (2) Low boiling alcohols, such as t-butanol, can be used as a solvent, and are much easier to remove than higher boiling DMF or DMSO. Commercialization of enzymatic synthesis will require a process that is simple, rapid, and cost-effective.

2.5.1 Monoacylglycerols (Monoglycerides)

Since monoacylglycerols are the simplest food surfactant molecules, researchers have investigated enzyme catalyzed esterification of glycerol with fatty acids (Hari-Krishna and Karanth 2002; Montero et al. 2003; Waldinger and Schneider 1996). Chou (2013) has disclosed lipase-catalyzed reactions of glycerol with fatty acids or their alkyl esters. The solvent is a lower secondary or tertiary alcohol. Thum et al. (2009) describe a batch tank reactor, that uses a gas stream for agitation and removal of water from direct esterification mixtures. The gentle agitation also avoids the size reduction of the catalyst support. Schoerken (2008) found that addition of alkaline salts of Group I and II metals significantly accelerated the reaction rates of enzyme esterification reactions.

2.5.2 Monoacylglycerol Derivatives

Derivatives of monoacylglycerols can be synthesized under mild conditions with natural enzymes. Citric, succinic, and acetic acids are chemically converted to their anhydrides to improve their reactivity, but this leads to lower yields. Enzymatic catalysis could make this step unnecessary. Huang, et al. (2012) investigated the reaction of citric acid and monoacylglycerols catalyzed by lipase, and using t-butanol as a solvent. Reaction rate increased with increasing temperature and up to a limiting value with increasing enzyme load. Fatty acid esters of diacetyltartaric acid have been prepared using transacylase or lipase enzymes (Aracil-Mira 2000).

2.5.3 Propylene Glycol Esters

Propylene glycol esters do not have the advantage of being widely occurring in nature. However, the regioselectivity and mild reactions of enzymatic reactions might still be attractive. Christiansen and Creemers (2008) produced 2-propylene glycol stearate by hydrolysis of propylene glycol distearate. However, the method as described would need improvements to be commercially viable. Chen et al. (2012) demonstrated that varying solvent composition can favor either the monoester or diester of 1,3-propylene glycol.

2.5.4 Saccharide Esters

Saccharides and fatty acids are well understood natural macronutrients. Esters formed using naturally occurring enzymes might have a reasonable case for a "natural" claim. Monoesters would have the highest HLB values and should be favored by enzymes that are selective for primary alcohols (Li et al. 2003). Ye and Haes (2012) have studied the reactions of saccharides and fatty acids, using immobilized lipase in stirred tank and packed bed bioreactors. Monoester/diester ratios ranged from 81:19 to 93:7. Particle size, also affect the reaction rates in these heterogeneous reactions. (Ye et al. 2014).

2.5.5 Polyglycerol Esters

Although polyglycerols are chemically produced at high temperatures, lipase enzymes can serve as a regioselective tool for producing esters under mild conditions. Linear polymers have primary alcohols at the two terminal carbon atoms. Charlemange and Legoy (1995) synthesized a surfactant with high monoester content using lipase in a solvent-free system. Experiments have also been carried out with polyricinoleic acid,

using *Candida antartica* (Hayes et al. 2012). Ortega et al. (2013) reacted polyricinoleic acid with polyglycerol, evaluating different enzymes and reaction conditions. Immobilized *Rhizopus orrhizus* was determined to be most cost effective, and a vacuum reactor with a gas sparge produced a product that met EEC standards.

2.5.6 *Lecithin*

A great deal of molecular design can be accomplished by a variety of specific phospholipases. Phospholipase A2 can be used to prepare lysolecithin (Hibino et al. 1991) or phospholipase A2 (Morgado et al. 1995). The reaction can be carried out in reverse to synthesize lysolecithin from glyceryl phosphatidylcholine and a fatty acid (Hibino et al. 1989). Transphosphatidylation may be accomplished using phospholipase D and a hydroxyl containing molecule (Masashi et al. 2005). Karuma et al. (2013) acetylated soy and egg lecithin using vinyl acetate and *Mucor mehei*. The method produces complete selective acetylation of phosphatidylethanolamine.

2.5.7 *Transglutaminase Modification of Dairy Proteins and Hydrocolloids*

As already discussed, proteins are macronutrients that can contribute surface activity. Transglutaminase can modify structure by cross-linking. The enzyme acts by intramolecular or intermolecular exchange of an amide with a primary amine. Milk proteins have been modified with transglutaminase (Yiiksel and Erdem 2009). Reaction rate for casein proteins are high, possibly due to its open structure. However, globular proteins, such as whey, can be modified to improve functionality by reaction with transglutaminase (Clare and Daubert 2010, 2011).

Protein-protein and protein-carbohydrate interactions are well known in the food industry (Gaonkar and McPherson 2006). Most of these interactions are due to electrostatic attraction/repulsion, Van der Waals forces, and hydrophobic bonding. Transglutaminase changes the positions of covalent bonds. Casein proteins can be incorporated into amine-containing hydrocolloids, such as chitosan (Song and Zhao 2013) or Konjak galactomannan (Yin et al. 2012).

2.6 Current Trends in Synthetic Methods

Since the synthesis of urea, reagents and techniques have been improved at an ever increasing rate. Many complex natural products have been prepared synthetically. Reagents and techniques to carry out regioselective and enantioselective reactions have made these syntheses possible. Sophisticated analytical methods were invented to measure these successes.

The forward path is to design synthetic strategies that have minimal impact on the environment. Biodegradable waste streams will be highly prized. Naturally occurring enzymes meet this need, and offer great energy savings. Commercialization has gone slower. Problems with mutual reactant solubility at ambient temperatures, and separation of products need to be solved. Conversion to enzyme technology requires capital investment in an industry which has low gross margins. Once these technical and economic problems have been sorted out, we can look forward to more nature-mimetic emulsifier syntheses.

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Chapter 3
Analysis of Food Emulsifiers

Gerard L. Hasenhuettl

3.1 Introduction

Food Emulsifiers are regulated by government agencies, such as the Food and Drug Administration. Regulations include composition, manufacturing methods and analytical specifications (Federal Register 2013). Analytical specifications may relate to performance in food applications. Non-governmental analyses are commonly negotiated between emulsifier suppliers and their customers. Analyses are usually carried out in the supplier's quality control laboratory, and the results stated on a Certificate of Analysis. The customer's laboratory may test to confirm the results. Disputes may be submitted to an independent laboratory for resolution.

Analytical tests of ingredients are important because once they are incorporated into a complex food product; separation and analysis are more difficult. Discussion of ingredients in food products is beyond the scope of this chapter, and is ably covered elsewhere (Cruz 2014).

Standardized procedures have been developed by professional societies, such as the Association of Official Analytical Chemists (Latimer 2012P (AOAC), American Oil Chemists' Society (AOCS) (Firestone 2013a), and American Society for Testing Materials (ASTM).

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3.2 Separation Methods

As noted in Chap. 2, commercial food emulsifiers are mixtures of Surface active molecules of varying polarities. This makes them good candidates for separation by extraction or absorption chromatography. The use of solvents creates a waste disposal problem, so efforts are being made to eliminate them, or minimize the scale of the process.

3.2.1 Thin Layer and Column Chromatography

Thin layer and column chromatography are based on differential adsorption of molecules as solutions as they move through a granular solid. Polar adsorbents interact more strongly with polar compared to non-polar solutes. Therefore, non-polar molecules will travel further on a TLC plate, or elute first from a packed column. For example, triacylglycerol will elute from a silica column with hexane, 5% diethyl ether in hexane will elute diacylglycerols, followed by 10% ether in hexane. A silver-impregnated Celite column accomplished the same separation using a single solvent composition (Dieffenbacher 1989). Thin-layer chromatography can also be used to separate emulsifier components. The adsorbents may be coated on glass or plastic plates, or glass rods. These techniques are quick methods for qualitative identification. One disadvantage is that compounds having the same RF value may obscure the results. Two-dimension TLC, using different solvents may overcome this obstacle.

Preparative thick-layer chromatography is an inexpensive method for separating lipid sample for further analysis; such as spectroscopy or mass spectrometry. Preparative high performance liquid chromatography has replaced preparative TLC in many laboratories.

Two-dimensional TLC has been used to separate the components of soy and egg lecithin, using acidic and basic solvents (Watanabe et al. 1986). Detection of these compounds may be achieved with a spray of sulfuric acid or dichlorofluorescein. However, phosphatides may be distinguished from other lipids by using selective reagents or spectroscopy (Senelt et al. 1986; Duden and Fricker 1977) Quantitative detection has also been carried out using a silica rod (Tanaka et al. 1979). Experimental design has also proven useful for optimizing separation of phospholipids (Olsson et al. 1990). In biological samples, phospholipids occur at low concentrations. High performance liquid chromatography, a more sensitive method, has largely replaced TLC for these analyses. HPLC, mass spectrometry, and their combinations will be discussed later in this chapter.

Monoacylglycerols may be modified by reaction with organic acids, as discussed in Chap. 2. TLC may be used to monitor these reactions and determine the composition of the products (Bruemmer 1971; Yusupoca et al. 1976; Jodlbauer 1981). Succinylated and lactylated monoacylglycerols (Schmidt 1976) as well as DATEM surfactants have been analyzed.

Molecules with multiple functional groups and polymeric head groups present a significant challenge. One method employed a Kieselgel G TLC plate, with a hexane/acetone/acetic acid solvent mixture (Regula 1975). Spots were visualized by spraying the plate with bromocresol green. Sucrose esters were separated by TLC (Li et al. 2002), and rod-TLC/flame ionization.

3.3 Wet Chemical Analysis

The earliest methods for analyzing fats, oils, and their derivatives were wet chemical procedures, that is, they utilized solvents and chemical reactions. These tests generally rely on titrations or calorimetry to determine the end point. For example, free fatty acid may be titrated with standard alkali in an alcohol solution. Wet chemical methods are time-tested, simple, and require inexpensive equipment. On the other hand, they are labor-intensive and require disposal or recycling of solvents. A number of these tests are being replaced by instrumental methods, which use auto sampling, digital data collection, and much less solvent.

3.3.1 α-Monoacylglycerols

Production of Monoacylglycerols (see Chap. 2) yields a 90/10 ratio of 1(α)-monoacylglycerols and 2(β)-monoacylglycerol positional isomers. The sn-1 and sn-3 positions on the glycerol backbone are chemically indistinguishable, while the sn-2 position is a secondary alcohol. Random distribution theory would predict a 2/1 ratio. The discrepancy may be rationalized by lower steric repulsion of a fatty acid esterified at a terminal position.

The 1(α)-monoacylglycerol has adjacent (vicinal) hydroxyl groups at the sn-2 and sn-3 positions. Reaction with the periodic acid cleaves the chain between the vicinal hydroxyl groups (see Fig. 3.1) in a standard analytical procedure (Firestone 2013b). The sample is reacted with an excess of periodic acid in a methanol solution. Potassium iodide is added, and the liberated iodine is titrated with a standard arsenite solution. In order to correct for the presence of free glycerol, the glycerol must be extracted and analyzed. The wet method is not suitable for mixtures that

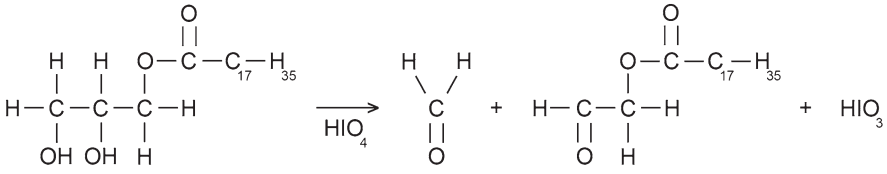

Fig. 3.1 Cleavage with periodic acid to determine α-monoglyceride

contain other molecules with vicinal hydroxyl groups. Since the majority of the monoacylglycerols occurs as the α-isomer, this method has been accepted as a specification. Due to the problem of solvent, the wet method has largely been replaced by instrumental methods that measure total monoacylglycerols.

3.3.2 Acid Value/Free Fatty Acid

Fatty acids are used as starting materials in the preparation of surfactants (see Chap. 2). During interesterification, small amounts of fatty acid may be split off. After neutralization, free fatty acids remain in the product. Since fatty acids impact functionality in a number of products, they must be held to a minimum value.

The acid value is determined by dissolving a weighed sample in a solvent and titrating with standard potassium hydroxide to a phenolphthalein end point (Firestone 2013c). In cases where the method is used to monitor acetic anhydride reactions, (DATEM or acetylated monoacylglycerols): an aprotic solvent must be used to prevent the anhydride reaction with alcohol. Potentiometric titration to an inflection point may also be used to determine the acid value. The acid value is calculated using the following equation:

$$\frac{(A-B)N}{56.1W}$$

A = ml KOH required to neutralize the surfactant sample
B = ml KOH to neutralize a blank
N = Normality of KOH solution
And 56.1 is the molecular weight of KOH

3.3.3 Iodine Value (IV)

The saturation or unsaturation of fatty acid side chains on surfactants determines the ability of the surfactant to crystallize or form complexes with other food ingredients. Reaction with iodine monochloride or iodine monobromide, followed by titration of excess iodine with standard thiosulfate, to determine the iodine value is an accepted method to define the level of unsaturation (Firestone 2013d). Iodine value is defined as the number of centigrams of iodine absorbed per gram of sample. The following formula is used to calculate iodine value

$$\frac{12.6N}{(S-B_0)W}$$

N = normality of thiosulfate solution
S = ml. solution to titrate the sample
B_0 = ml. solution to titrate a blank
W = weight of sample

When reporting the iodine value, it is important to also include the method used.

Instrumental methods, such as gas-liquid chromatography and infrared spectroscopy, have been developed to measure iodine value.

3.3.4 Peroxide Value (PV)

As mentioned in previous sections, surfactants containing double bonds are susceptible to oxidative degradation (rancidity). The initiating step in the chain reaction is insertion of oxygen into a carbon-hydrogen bond to form a hydroperoxide. Surfactants which have been bleached, such as sorbitan monostearate or sodium stearoyl lactylate, may contain residual peroxide. These species are potential initiators of the oxidative chain reaction.

Peroxides and hydroperoxides are determined by treating a weighed sample with an excess of potassium iodide in an isooctane solution (Firestone 2013e). Iodine, which is liberated by the reaction, is titrated with standard thiosulfate solution to an end point using starch as an indicator. Precautions must be taken to ensure that glassware is free from residues from oxidizing or reducing agents. Strong ultraviolet light must be avoided because of its potential to promote photochemical oxidation. Peroxide value is defined as the number of miliequivalents of peroxide per kilogram of sample. It is expressed in the formula:

$$\frac{1000VT}{\mathrm{M}}$$

V = volume of the titrant solution
T = normality of thiosulfate solution
M = weight of the sample

Recently, high performance liquid chromatography (HPLC) has been used to determine peroxide value.

3.3.5 Saponification Value

As with all esters of carboxylic acid, cleavage of the ester bond may be accomplished by reaction with alkali and water to produce a salt of the fatty acid and an alcohol. This reaction is known as saponification. The saponification value is defined as the number of milligrams of potassium hydroxide required to saponify one gram of sample (Hummel 2000a; Firestone 2013f). A weighed sample is reacted with standard alcoholic potassium hydroxide and the excess alkali is titrated with hydrochloric acid solution to a phenolthalein end

point. Alternatively, a potentiometric titration may be used if a visual end point is difficult to see. The saponification value is calculated using the following formula.

$$\frac{56.1\mathrm{N}(\mathrm{S}-\mathrm{B})}{\mathrm{W}}$$

S = ml required to titrate a sample
B = ml required to titrate the blank
N = normality of the reagent (56.1 is used for KOH)
W = weight of the sample

When comparing triacylglycerol's, the saponification value varies with the chain length of the fatty acid. Shorter chains give higher values while longer chains produce lower values. For surfactants, the saponification value is affected by both chain length and the degree of substitution. Shorter fatty acids and higher degrees of substitution yield higher values, while longer chains and lower degrees of substitution produce lower values.

3.3.6 Hydroxyl Value

When polyols are esterified, some hydroxyl groups remain unreacted. The concentration of these free hydroxyl groups may be determined by reacting acetic anhydride in the presence of pyridine. The sample is treated with water and heated to hydrolyze unreacted anhydride to acetic acid. The acetic acid is titrated with standard alkali with an indicator to determine the end point. The hydroxyl value is defined as the number of milligrams of potassium hydroxide equivalent to the hydroxyl content of one gram of sample (Hummel 2000b; Firestone 2013g). It is calculated using the formula:

$$\frac{56.1\mathrm{T}\left(\mathrm{V}-\mathrm{V}_0\right)+\mathrm{A}}{\mathrm{M}}$$

T = normality of potassium hydroxide (56.1 = mol. Wt. of KOH)
V = ml required to titrate sample
V_0 = ml required to titrate a blank
W = wt. of sample in g
A = acid value

The hydroxyl value is an indicator of the number of free hydroxyl groups in the molecule, and therefore the hydrophilic character of the surfactant. High hydroxyl values indicate high HLB, and low values indicate low HLB.

The reaction and titration procedures are time consuming and require a great deal of skill on the part of the analyst. Minor variations in the procedure can cause large discrepancies in the results. It is therefore recommended that the hydroxyl value be reported as an average of duplicate samples. Efforts have been made to correlate

hydroxyl value with instrumental methods, such as near-infrared reflectance spectroscopy (NIR).

3.3.7 Lactic Acid Analysis

Lactic acid is used in the manufacture of surfactants, such as lactylated monoacylglycerols or propylene glycols, sodium stearoyl lactylate (SSL), and calcium stearoyl lactylate (CSL). Lactic acid in these products occurs in two forms: free and esterified. Total lactic acid is the sum of these two forms. Lactic acid is a bifunctional molecule which can self-condense and form polymers. Total lactic acid has been determined by reaction of a weighed sample with alcoholic potassium hydroxide, neutralization with hydrochloric acid, extraction with diethyl ether, and titration with standard potassium hydroxide. Free and polylactic acid and water insoluble combined lactic acid (WICLA) are determined by dissolving a weighed sample in benzene. The aqueous extract is titrated with potassium hydroxide to determine the free acid. The benzene layer is dried and titrated with potassium hydroxide in the same manner as for total lactic acid. Two problems with these methods are the laborious extraction and separation steps, and the use of carcinogenic benzene.

A modification of the method uses a chloroform/petroleum ether mixture for the determination of lactic, as well as citric and diacetyltartaric acid species (Franzke 1977). The same author reported that enzymes could be used instead of potassium hydroxide to cleave the ester bonds in the determination of total lactic acid (Franzke and Kroll 1980).

3.3.8 Reichert-Meisel Value

Some fats and oils, such as butter and coconut oil, contain short chain fatty acids (C4-C10). The Reichert-Meisel value was developed to measure content of these acids (Firestone 2013h). The method has been used to measure the concentration of acetic and tartaric acid esterified to monoacylglycerols. A weighed sample is reacted with an alkali solution, followed by neutralization with dilute sulfuric acid. Liberated acetic acid is distilled and titrated with standard alkali solution to a phenolthalein end point. The method is equipment intensive, since the distillation apparatus must be cleaned or replaced between analyses. The distillation step is also time consuming.

3.3.9 Determination of Moisture

Residual water is generally an undesirable contaminant in food surfactants. It can promote the growth of mold or other microorganisms. It can also result in ester cleavage, which increases the concentration of free fatty acids (hydrolytic

rancidity). Moisture may be picked up when surfactants are pumped through pipes that have not been adequately dried, or when they are flaked or beaded in high humidity conditions.

In a few cases, water is deliberately added to surfactant products. Small amounts of water in polysorbates inhibit phase separation. Water can form gels with some surfactants, which can improve functionality in certain applications. In any case, it is important to know the water concentration.

An old method to determine moisture was to drop some molten product on a hot plate. If there was no spatter, moisture was considered to be acceptable. In a more quantitative test, water was distilled from the sample and weighed. These older methods were unable to distinguish between water and other volatile components. Another method dissolves a sample in toluene, and distills water and toluene into a graduated receiver. The water is quantitated by volume. These distillation methods are labor and equipment intensive.

Titration with Karl Fisher reagent (SO2/I2/pyridine/2-methoxyethanol) has been developed for commercial fats and oils (Firestone 2013i), and lecithin (I), industrial oil derivatives (Firestone 2013j). Autotitrators are commercially available, which can process large numbers of samples without the need for cleaning between samples. Some impurities, such as peroxides, can react with Karl Fisher reagent to give high results. Near infrared spectroscopy (NIR) has been used to detect moisture in raw materials.

3.3.10 Soaps of Fatty Acids

Calcium and sodium salts of fatty acids (soaps) are formed in surfactants by alkaline catalysts during the manufacturing process. Inadequate neutralization post-reaction results in an unacceptable level of soaps. This can result in degradation of the surfactant, due to disproportionation reactions, especially during molten storage.

One analytical method consists of dissolving a weighed sample in an organic solvent/water mixture, followed by titration with standard hydrochloric acid solution (Firestone 2013k). Although the scope of this method is limited to analysis of fats and oils, it may be adapted for use with surfactants. Bromothymol blue or phenolphthalein may be used as an indicator. The method may also be adapted to potentiometric titrations. An alternative procedure to determine whether neutralization is complete is to measure the pH. A 5% solution of the surfactant is allowed to equilibrate to room temperature, and the pH is measured with a standard electrode. Values between 6.5 and 6.8 indicate the absence of soap, and adequate neutralization.

3.3.11 Phosphorus and Phospholipids

Soy lecithin is a widely used naturally occurring surfactant, which is obtained as a byproduct of soybean oil refining. Structurally, the molecule consists of phosphoric acid, esterified with two fatty acids, and an organic base or inositol (see Chap. 2).

Diacylglycerol phosphate has a similar structure, but lacks the organic base or inositol.

One way to determine the concentration of lecithin is to analyze for phosphorus and then apply a gravimetric factor. A titrimetric procedure saponifies a sample, followed by precipitation with a molybdate solution. The precipitate is washed and dissolved in an alkali solution. Excess alkali is then titrated with a standardized acid. Another method involves ashing a sample, dissolving the ash in acid, and determining the phosphorus colorimetrically (Firestone 2013l).

A similar, albeit less precise, technique is to precipitate the lecithin with acetone. The precipitate is dried and weighed. When soybean oil has been enzymatically refined, these simple methods are less accurate, due to the variable cleavage of fatty acids. Because of the importance of phospholipids in lipid metabolism and membrane structure, a great deal of effort has been expended to develop more precise quantitative methods. Techniques such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), mass spectrometry (MS), and HPLC/MS are discussed later in this chapter.

3.4 Measurement of Physical Properties

Physical properties of food surfactants often play a significant role in the appearance, texture, and flavor release of finished food products. Chapter 6 will discuss the physical and polymorphic properties of surfactants in greater detail. In this section we will survey some methods for measuring physical properties.

3.4.1 Color

Although color may be considered a physical property, its origin is related to the chemical composition of the starting lipid. Fats and oils contain minor amounts of tocopherols, tocotrienols, carotenoids, and chlorophyll. These compounds may be removed during processing, but may be "locked in" if the oil has been thermally abused. Side reactions that occur during processing may also result in dark colors. For example, carrying out an interesterification at too high a temperature can cause caramelization of sugar. Dark colors may not only affect the appearance of foods, but may also be an indication of other problems, such as oxidation or burnt off-flavors. The lightest color is therefore a quality goal.

Most dark colors originate in starting fat/oil starting materials. Strict receiving specifications must be adopted and consistently met. Color has traditionally been measured by comparison of a sample to a set of standard materials such as colored glasses. A widely accepted measurement in the oil processing industry is the Lovibond method (Firestone 2013m). A column of liquid or molten sample in a glass tube is observed over a white background and compared to a set of colored glasses. Values are determined for red® and yellow (Y) which arise from minor constituents in vegetable oils. An alternative test is the Gardner method (Firestone 2013n). This

procedure is used for crude lecithin and industrial oils. An automated method has also been adopted (Firestone 2013o).

Another comparative test is the FAC method (Firestone 2013p), which is a method that is used to analyze samples that are too dark to be read by the other methods. The weakness of all the comparative tests is that they are subjective judgements that require an experienced analyst. Spectrophotometric methods in the ultraviolet and visible regions are more objective (Firestone 2013q).The spectra are more versatile, since they can detect colors in addition to red and yellow (for example, green color due to chlorophyll).

3.4.2 Refractive Index

Clear liquids refract light because the speed of light is different in different media. Refractive index is the ratio of the speed of light in air to the speed of light in the liquid. Measurements are carried out in a refractomer (Firestone 2013r). It is commonly used to measure rates of reactions, such as hydrogenation and polymerization. The refractive index is correlated to a chemical property, for example, the concentration of hydroxyl groups or double bonds. In the polymerization of glycerol, refractive index increases with the degree of polymerization. The reaction end point is rapidly determined and then confirmed by the more time-consuming hydroxyl value.

3.4.3 Melting Point

Fats, oils, and their derivatives are heterogeneous mixtures of molecules, and therefore do not have sharp melting points as do pure homogeneous substances. Rather, a broad melting range is observed. To further complicate the matter, polymorphic crystalline forms may melt and recrystallize into a different polymorphic form during the range of increasing temperature. However, melting behavior is often critical in food applications. For example, the melting temperature of a peanut butter stabilizer must be matched to the fill temperature in order to prevent separation. This poses a significant challenge to melting point methodology.

A number of tests have been developed to describe the melting behavior of fat-based ingredients in diverse food applications. Capillary methods have long been used to characterize organic compounds (Firestone 2013s). Because of the broad melting ranges for fats, fatty acids, and their derivatives, the capillary method gives a range which is often subjective. Other tests have been designed to yield a single value for the melting point. The slip point is useful to obtain a melting point that is especially useful for dark-colored samples or ingredients that contain suspended materials (Firestone 2013t). The test, also known as the softening point, is carried out in an open capillary tube. A sample is heated at a programmed rate, and the slip point is defined as temperature at which the sample falls out of the tube. This value will be lower than that from a closed tube test, because it measures the first onset of melting.

The Mettler dropping point is a test which depends less on the observation of an experienced analyst (Firestone 2013u). A standard-sized disk of sample is placed in the Mettler apparatus and then is heated at a programmed rate. When the sample melts, it drops through a sensor, and the temperature is automatically recorded.

3.4.4 Viscosity

Viscosity of food surfactants is critical to material transfer in the manufacturing process. If a raw material or product is too viscous, it is difficult to pump it through a pipe, particularly in cold weather. It is generally used as a quality control specification for viscous liquids, such as polyglycerol esters and lecithin. This property is temperature dependent. Viscosity decreases as temperature increases. Materials may need to be heated in order to pump them through heat-traced pipes. One early method to measure viscosity is the bubble-time method (Firestone 2013v). A sample is poured into an ASTN tube in a constant temperature bath. The tube is then inverted and the time, in seconds, for the bubble to reach the closed end of the tube is measured. A formula is used to calculate the viscosity. A Brookfield rotary viscometer may also be used for measuring viscosity (Firestone 2013w). In this method, a liquid is filled into a gap between two concentric cylinders. The inner cylinder is rotated, and the strain is measured. Viscosity is calculated using a formula.

3.4.5 Specific Gravity

Specific gravity is used to convert weight and volume measurements.

For example, a batch sheet may call for a liquid measured in pounds or kilograms. If the ingredient is pumped through a meter, the weight must be converted into volume. Specific gravity is also necessary to determine the volume of a package that holds a certain weight of liquid.

Specific gravity is measured by filling a tared pycnometer with liquid at 25 °C., and weighing the sample. If the sample is not liquid at this temperature, 40 or 60 degrees may be used (Firestone 2013x). A method is also available to measure the specific gravity of a solid (Firestone 2013y). Care must be taken to remove air from the samples, since it can give erroneously low results.

3.5 Instrumental Methods of Analysis

Developments in analytical chemistry have enabled the development of sophisticated instruments that may be applied to analysis of lipids. Instrumental methods have advantages over wet chemical titrations: (1) more information can be obtained

about composition and structure of surfactants, (2) Less waste disposal (or recycling) of solvents and reagents, (3) Automation of sample preparation, data collection, and archiving results. The greatest obstacle to implementation is the high cost to purchase and maintain the instruments and peripheral equipment.

3.5.1 Thin Layer Chromatography (TLC)

Thin-layer chromatography was discussed earlier as a means to separate and identify mixtures of surfactants. These methods are not well suited for quantitative analysis, but are very simple to carry out. Coated rods may be used for separation, and the bands quantitated by scanning the rod with a flame ionization detector. Instrumental methods for thin-layer chromatography have recently been more fully discussed (Poole 2014).

3.5.2 Gas-Liquid Chromatography (GLC)

Gas-liquid chromatography vaporizes a sample and passes it through a column packed with adsorbent at high temperature. Polar or reverse-phase adsorbents may be used. Separation depends on polarity and molecular weight of the surfactant. Detection of component peaks may be accomplished with thermal or flame ionization detectors. A mass spectrometer may be used in combination with gas-liquid chromatography (GLC/MS) to give detailed structural information about each peak.

Analysis of lipids by GLC is challenging, due to their high molecular weight and low volatility. Chemical reactions of lipids to yield more volatile derivatives have been successful. However, these chemical reactions add an additional step to the analysis.

The most common analysis is the determination of fatty acid composition. Fatty acids are cleaved from their esters and reacted with a derivatizing reagent to produce a volatile derivative. The derivative is injected into the GLC, and separated on a packed or capillary column (Firestone 2013z). The chain length and degree of unsaturation of the fatty acid determine the strength of adsorption. The retention times of the peaks are recorded and correlated to previously analyzed or internal standards. Concentrations are determined by peak heights or areas, and corrected by the response factor for each peak. Mono- and diacylglycerols are the emulsifiers with the simplest compositions. A dry sample is reacted with chlorotrimethylsilane and hexamethylsilazane in the presence of pyridine (Nakanishi and Tsuda 1983; Bruschweiler and Dieffenbacher 1991; Firestone 2013aa). GC/MS analysis of mono- diacylglycerols has also been reported (Lee 1988). Two-dimensional GLC coupled with time-of-flight mass spectrometry (GCxGC-TOF-MS) has been used for regiospecific analysis of mono/diacylglycerols from glycerolysis reactions (Indrastri 2010).

The method may also be used to analyze mixtures of propylene glycol esters and mono- and diacylglycerols. Figure 3.2 show a typical separation. Monomargarin (a C-17 monoacylglycerol) has been used as an internal standard. Unfortunately, it is time-consuming to synthesize, expensive to purchase, and has limited stability. (+/−)-Batyl alcohol, iso-atomic with monomargarin, has been shown to work as an alternative standard (Hasenhuettl et al. 1990). Since batyl alcohol is an ether, it is more stable than a glyceryl ester.

Polyol distributions of food surfactants can be determined by cleaving the ester linkage by saponification, followed by analysis of the polyol fraction. If the polyols are not sufficiently volatile or are unstable at high temperatures, they may be converted to trimethylsilyl ethers. For example, sorbitol, sorbitan, and isosorbide, cleaved from their fatty acid esters, can be determined by GLC (Murphy and Grislett 1969; Tsuda et al. 1984). Glycerol through dodecaglycerol, isolated from polyglycerol esters of fatty acids, may be determined by GLC analysis of their trimethylsilyl ethers (Schutze 1977). Supercritical fluid chromatography (Macka et al. 1994) and a combined GLC/HPLC method (DeMeulenaer et al. 2000) have been used to obtain polyol distributions. High temperature GLC has been carried out to determine the distribution of polysorbates (Lundquist and Meloan 1971; Kato et al. 1989). Although sucrose esters of fatty acids decompose at high temperatures, they have been analyzed by GLC (Karrer and Herberg 1992). Mass spectrometry was utilized to establish identification of the eluted peaks, and supplied additional information (Uematsu et al. 2001).

GLC is also a valuable tool to detect contaminants, such as heat exchange fluids (Firestone 2013ae).

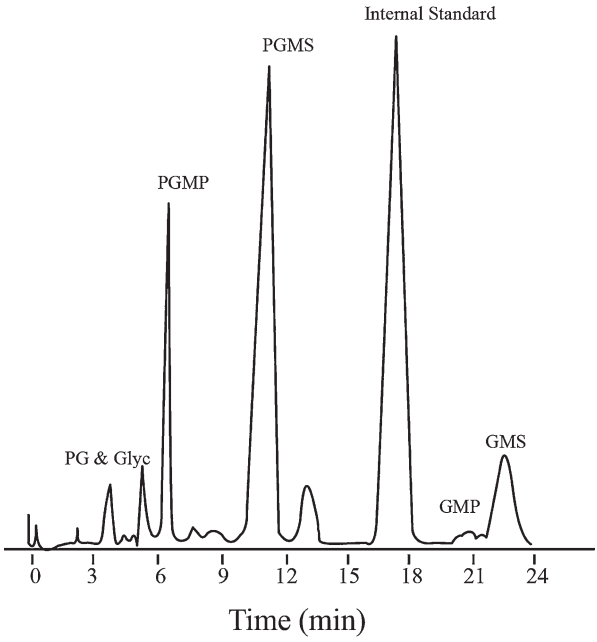

Fig. 3.2 Determination of monoacylglycerols and propylene glycol monoesters by gas-liquid chromatography (GLC) using batyl alcohol as an internal standard, modified from Hasenhuettl et al. (1990)

3.5.3 High Performance Liquid Chromatography (HPLC)

HPLC is a logical extension of column chromatography. It is a very useful technique for quantitative determination of lipids and their derivatives (Christie and Ha 2010; Fanali et al. 2013). Because of their low volatility a sample is injected into an HPLC, and the solvent carries it through the column and into the detector. Over time, column sizes have been reduced to minimize the sample size and solvent volume. The nature of the column determines the mode of separation. For example, a standard silica column separates the components by adsorption of the polar functional groups. Non-polar (reverse phase) columns, such as polystyrene, cross-linked with divinylbenzene, adsorb lipophilic regions of the molecules. When both are used with a single sample, complementary information is obtained. A size-exclusion column separates molecules by shape and molecular weight.

One problem with using HPLC with lipids is their poor response to conventional detectors. Saturated lipids do not absorb ultraviolet light at a unique region of the spectrum. A refractive index (RI) detector may be used, but it is much less sensitive, and may only be used for isocratic (single solvent) separations. An evaporative light scattering (ELSD) detector was developed to overcome these problems (Christie 1992; Hammond 1993; Lee et al. 1993). Solvent is flash-distilled in the detector, and the residual non-volatile matter scatters light and is recognized as a peak. Figure 3.3 shows a separation of phospholipids using this detector. A charged aerosol detector (CAD) has also been shown to profile phospholipids after enzymatic transphosphatidylation reactions to produce phosphatidylinositol (Iwasaki et al. 2013).

Perhaps the most common food surfactants analyzed by HPLC have been the monoacylglycerols (Filip and Kleunova 1993; Firestone 2013ab; Takagi and Ando 1994; Ranger and Wenz 1989; Tajano and Kondoh 1987) and Phospholipids (Christie 1996; Melton 1992; Sotirhos et al. 1986; Hurst and Martin 1984;

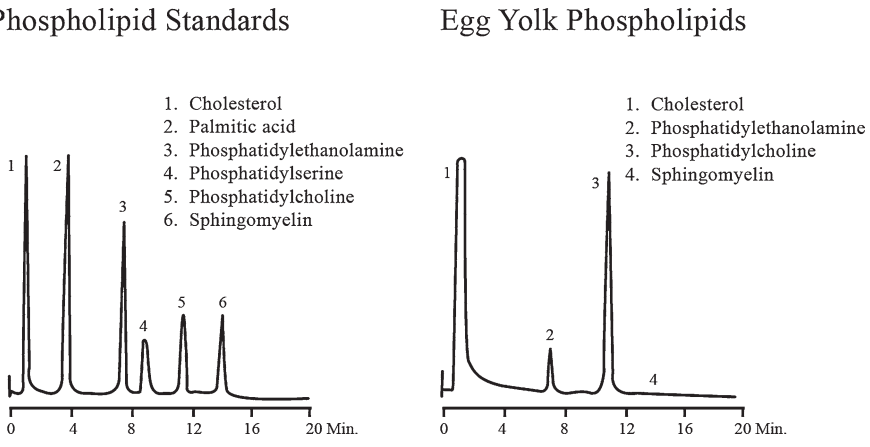

Fig. 3.3 Determination of phospholipids using high-performance liquid chromatography (HPLC) and an ELSD detector, adapted from Alltech Inc. brochure

Huyghebaert and Baert 1992; Tumanaka and Fujita 1990; Rhee and Shin 1982; Hsieh et al. 1981; Kaitaranta and Bessman 1981); (Firestone 2013ac; Luquain 2001). Free glycerol may also be determined by HPLC (Firestone 2013ad).

Polyglycerol mono- and polyesters have been separated on a Li-Chromosorb column (Garti 1981; Kumar et al. 1984). Sorbitan esters of fatty acids have also been separated on the same stationary phase (Garti and Acerin 1983). Sucrose esters of fatty acids were determined using their 3, 5-dinitrobenzyl derivatives (Murakami et al. 1989). Determination of propylene glycol alginate in aqueous systems has been accomplished by high performance anion exchange chromatography (Diepenmaat-Diepenmaat-Walters et al. (1997). Contamination of lipid derivatives by heat exchange fluids have been analyzed by HPLC (Firestone 2013ae).

3.5.4 Mass Spectrometry

Mass spectrometry (MS) has been a powerful tool for determining the structures of organic molecules (Gross 2011). Molecular, or parent, ions indicate the molecular weight of the molecule. Fragments or daughter ions provide evidence of substructure and functional groups. Tandem mass spectrometry allows the identification of molecular and fragments in a single sample. An application to lipids has been slowed by two factors: (1) Lipids are not volatile and not amenable to injection into a high vacuum instrument; (2) lipids are usually a mixture of molecules that produce a bewildering combination of molecular and fragment ions. Development of new sample introduction methods and combination with HPLC has overcome these obstacles in many cases. Soft ionization methods, such as atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), electrospray ionization (ESI), and matrix assisted laser desorption ionization (MALDI) have allowed great advances in characterization (Cai and Syage 2006; Byrdwell 2005a, b, c; Murphy and Gaskell 2014). ESI is useful for lipids over a wide range of molecular weights. Non-polar lipids with low molecular weights are more amenable to APPI. In an emerging field, known as lipidomics, a complex mixture of lipids can be injected directly into a mass spectrometer, and characterized by a wide variety of ionization methods (Han and Gross 2005; Ekoos 2012).

Phospholipids are distinguished from other lipids using their lithium salts and the nitrogen rule. Fast atom bombardment (FAB) was used to characterize the phospholipids from egg yolk (Trautler and Nikiforov 1984). Protonated molecular ions MH+ were easily resolved and identified polysorbates in foods were characterized by negative ion MS (Daniels et al. 1985). Two families of peaks were identified: free polyoxyethylene and polyoxyethylene esterified to sorbitans. Fatty acids esterified to sorbitans could also be identified in the spectrum. MALDI time of flight MS was also reported as a method for analysis of polysorbates (Frison-Norrie 2001).

Sucrose esters of fatty acids were analyzed by ESI-MS (Schuyl and VanPlaterink 1994). This technique showed a family of molecular ions corresponding to degree of esterification of fatty acids to sucrose.

Electrospray ionization tandem mass spectrometry was able to detect 30 species of glycerol oligomers (n = 1–6), esterified with ricinoleic acid and RA oligomers (Orfanakis et al. 2013).

3.5.5 High Performance Liquid Chromatography/Mass Spectrometry (HPLC/MS)

Integration of HPLC and MS had been difficult because of the problem of removing large volumes of solvent prior to injection into the mass spectrometer. Early efforts collected fractions from the HPLC, evaporated the solvent, and injected the residue directly into the MS ionization source. Development of microbore columns and direct injection techniques, such as APSI and ES, allowed the marriage of the two technologies.

When ELSD, a destructive detector, is used, a split stream is used to protect the MS injector. Normal phase HPLC, coupled with ESI tandem mass spectrometry (MS/MS), is useful for separation and characterization of complex phospholipid mixtures (Larsen and Hyattumff 2005). Phospholipids are separated by head group class, and molecular weights of the class members were determined by MS. Additional information could be obtained by collecting the fractions from normal phase HPLC using reverse-phase HPLC to separate class members, and find detailed structure information by MS (DeMeulenaer et al. 2000). In this case also, additional detail could be obtained by a combination of normal and reverse-phase HPLC. Glycolipid biosurfactants have been characterized by HPLC/MS (Nunez et al. 2005). Because the head groups of these molecules are large and complex, the methodology may be useful for other high HLB surfactants, such as polysorbates, polyglycerol esters, and sucrose esters.

Because normal phase and reverse-phase chromatography are orthogonal methods, coupling them both simultaneously to MS (HPLC/2MS), has been developed as a useful separation method for complex lipid-phospholipid mixtures (Byrdwell 2005c). APCI and ESI are also complementary techniques. Coupling of all four methodologies (HPLC2/MS2) is capable of generating massive amounts of compositional and structural data simultaneously. Although HPLC/MS methods provide detailed information, they are too expensive to be used for quality or process control in surfactant manufacturing. However it is being used in research laboratories in large companies and universities.

3.5.6 Spectroscopic Methods

As previously noted in our discussion of HPLC detectors, saturated fatty acid chains do not absorb in significant regions of the ultraviolet spectrum. However, functional groups can form colored complexes with a number of reagents. Measurement of the

ultraviolet absorption in a spectrometer can be correlated with the concentration of the surfactant. Anionic surfactants form complexes with methylene blue, which may be detected at 650 nm. Cationic surfactants react with Orange 2 to yield a complex which is detectable at 485 nm (Lew 1975). DATEM meta-vanadate complex can be detected at 490 nm (Schmidt 1979). Phosphatidylcholine in lecithin can complex with methylene blue (Hartman et al. 1980), dipicrylamine (Mueller 1977), or Reinecke's salt (Moelering and Bergmeyer 1974), for spectrophotometric analysis. Total phosphorus content can be derived from the phosphomolybdate analysis (Firestone 2013af).

Polyoxyethylene chains can form colored complexes, which can then be determined spectrophotometrically (Kato et al. 1989). Polysorbates have been analyzed by this method in a variety of food products (Daniels 1982; Saito et al. 1987; Tonogau et al. 1987).

In contrast to the UV spectrum, the infrared spectrum has a number of wavelengths which are diagnostic of functional groups that are present in surfactants (Tasumi 2014); in particular, carbon-carbon double bonds, carbonyl, hydroxyl, amine, and phosphate groups. Carbonyl and hydroxyl stretching bands have been useful for qualitative and quantitative analysis of polysorbates that were determined by other methods (Kato et al. 1989). Near-infrared (NIR) spectroscopy has been used to determine the iodine value by measuring carbon-carbon double bond frequencies. A calibration curve is used to correlate the NIR method with the wet method (Firestone 2013ag). NIR was also used as a quick method to determine hydroxyl values for polyglycerols and polyglycerol esters by measuring the O-H stretching frequency (Ingber 1986). FT-IR spectrometry may also be used to detect impurities in surfactants and lipids. An alternative to a titrimetric determination of peroxide value is to measure iodine, liberated by peroxide with a spectrophotometer (Yamanaka and Kudo 1991). Residual dimethylformamide in sucrose fatty acid esters has been determined by the absorption peak at 1675 cm^{-1} (Jakubska et al. 1977). However, this technique is not sufficiently sensitive to detect impurities at the ppm level.

NIR and Fourier transform infrared spectroscopy (FTIR) have a great advantage of obtaining compositional values rapidly. This provides the opportunity to measure the progress of chemical and enzymatic reactions in real time and serve as a process control. For example, the esterification of fatty acids and glycerol can be monitored by NIR (Blanco et al. 2004). FT-IR is also useful for the determination of hydration (Pohle et al. 1997), bilayer geometry and metal binding (Grdadolnik and Hadm 1993) and phospholipids. FT/NIR has been used to measure acetone-insoluble material, acid value, and moisture in lecithin, obtained from soybean oil processing (Li et al. 2009).

Atomic absorption spectroscopy (AA) is useful for detection of metals in surfactants and lipids. Heavy metals, such as lead (Firestone 2013ah), or pro-oxidants, such as iron, copper, or chromium (Firestone 2013ai) can be detected. Other metals detected are sodium, calcium, magnesium, nickel, silicon, and chromium (Firestone 2013aj).

3.5.7 Nuclear Magnetic Resonance

Atoms having an odd atomic number display a magnetic resonance, which is influenced by its chemical environment (Hore 2015). Measurements may be carried out by placing a dissolved sample in strong electromagnetic and radio frequency fields. The magnetic field is varied (swept), and peaks are observed by a radio frequency detector. Locations of peaks are determined by the atoms to which the nucleus is bonded. Splitting patterns are observed when adjacent atoms are magnetically susceptible (have an odd atomic number).

Wide line (low resolution) NMR is frequently used to determine the Solid Fat Content (SFC) of the sample (Firestone 2013ak). This method is limited to shortenings, margarine fats, cocoa butter, and other solid fats, which may contain surfactants.

Chemical shifts in NMR peaks have been used to identify surfactant mesomorphic phases in aqueous solutions (Lindblom 1996). Mesomorphic phases will be discussed further in Chap. 6. Proton (1H) NMR is the oldest method applied to organic molecules. However, because of the large number of hydrogen atoms present in alkyl side chains, it has very limited use in lipid applications. Phosphatidylcholine content has been determined by measuring the choline protons at 3.3 ppm (Press et al. 1981); (Kostelnik and Costellano 1973). Measurement of the vinylic protons at 5.5 ppm has been proposed as an alternative to the titrimetric determination of iodine value (Sheeley et al. 1986).

Chemical shifts of carbon (^{13}C) are sensitive to the presence of adjacent functional group. For example, a carbon in a carbonyl group will have a much different chemical shift than a carbon in a methyl group. Since there are many fewer carbon atoms than hydrogen atoms in lipids, spectra are much simpler and easier to interpret (Gunstone 1993). Glycerol carbon atoms were used to determine concentrations of monoacylglycerols, diacylglycerols, and free fatty acids in olive oil (Sacchi et al. 1990). Regio- and stereoselectivity of monoacylglycerols derived from enzymatic reactions can also be established (Mazur et al. 1991). Chemical shifts for diagnosis of carbon atoms in monoacylglycerols, propylene glycol esters, acetylated monoacylglycerols, phosphatidylcholine, and phosphatidylethanolamine are shown in Table 3.1. ^{1}H and ^{13}C NMR have been studied to determine multilamellar (Everts and Davis 2000) polyglycerols (Istratov et al. 2003), and polysorbate 60 (Dang 2006). Phosphorus (^{31}P) NMR is a very useful technique for determining structure and concentration of phospholipids (Glonek and Merchant 1996; Gillet et al. 1998). Since there is only one phosphorus atom per molecule, peak assignment is straightforward compared to ^{1}H and ^{13}C NMR. This is somewhat offset by the numerous phosphorus-containing molecules occurring in nature. Optimization of solvent systems for best resolution was reported (Bosco et al. 1997). Phospholipids in milk fat globule membrane have been characterized by ^{31}P NMR (Murgia et al. 2003).

1-D and 2-D NMR have been used to detect ricinoleic acid (RA) and oligomers of RA (Orfanakis et al. 2013). ^{1}H and ^{13}C NMR analysis of PGPR detected the presence of glycerol and its oligomers, esterified by ricinoleic acid and, to a lesser extent by other fatty acids.

Table 3.1 ^{13}C Chemical shifts (ppm) for some food surfactants

Surfactant Structure	Gl-1	Gl-2	Gl-3	N-CH_3	O-CH_3
Soy phosphatidylcholine	63.01	70.51	63.33	66.26	59.34
Egg phosphatidylcholine	62.94	70.63	63.78	66.62	50.32
Soy phosphatidylethanolamine	62.81	70.59	64.07	40.69	62.08
Egg phosphatidylethanolamine	62.81	70.59	64.07	40.59	62.13
1-Monoacylglycerol	65.04	70.27	63.47	–	–
1,2-diacylglycerol	65.04	72.25	61.58	–	–
1-propylene glycol monoester	69.46	66.13	19.2	–	–
2-propylene glycol monoester	65.92	71.77	16.25	–	–
Propylene glycol diester	65.42	67.98	16.5	–	–
Monoacetylated monoester					
A	62.07	72.89	61.40	–	–
B	63.00	68.19	65.26	–	–
Diacetylated monoacylglycerols	62.00	69.16	62.33	–	–

Adapted from Gunstone (1993)

3.6 Setting Specifications

The practice of setting specifications for food ingredients may be a matter of custom, such as adopting the supplier's values, or it may be a well-reasoned approach based on end product functionality. When developing new products, which are similar to existing products, the first practice may be perfectly acceptable, and even time-saving. Manufacturers of food ingredients are generally knowledgeable in applying their products in a variety of processed foods. Sometimes, a food processor may develop a new-to-the-world product, which has no analogous item currently on the market. In this situation, a logical, data-based approach is preferable.

It must be recognized, that when designing a known or analogous product, that there may be differences between surfactant suppliers, even if though their products meet the same range of analytical constants. This is particularly true of the more complex surfactants, such as polyglycerol esters, sorbitan esters, polysorbates, and phospholipids.

For new-to-the-world products, the first step in product development is to determine which attributes are critical to consumers. This is done using quality descriptor analysis techniques, focus groups, and consumer sensory analysis panels. Ingredients that enhance these attributes can then be investigated. Before testing food surfactants, the government food and labeling regulations which apply must be carefully reviewed. For example, does the product need to be Kosher or all natural? Is the ingredient permitted for use in the new product?

Once the ingredients have been identified, a statistical design should be constructed to optimize the critical attributes. Since ingredient interactions are well known (Gaonkar and McPherson 2005), the initial design, should be full factorial. Once all one- and two-factor interactions have been identified, a fractional factorial design can be developed to reduce the number of experiments. When optimal sur-

factant composition has been identified, the range of acceptable analytical constants (e.g. monoacylglycerols content or free fatty acid) must be defined. These values, along with analyses for absence of contaminants, are written into a raw material specification. The food processor and the surfactant supplier should confer to determine whether these specifications can be consistently met. The historical record of the suppliers' analyses should fall within the 95% confidence level. Failure to reliably meet these standards could result in returned surfactant shipments, production delays, or even product recalls. It may be necessary to revisit the product design in order to make it more robust.

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Firestone D (2013e) Method Cd 8b-90: peroxide value using isooctane; Ja 8-87: peroxide value of lecithin
Firestone D (2013f) Method Cd 3c-91: saponification value of fats and oils, modified using methanol
Firestone D (2013g) Method Cd 14-40: hydroxyl value of fats and oils (acetyl value); cd 13-60: hydroxyl value of fatty oils and alcohols
Firestone D (2013h) Method Cd 5-40: Reichert-Meisel value
Firestone D 2013i) Method Ca 2e-84: moisture by Karl Fischer method in fats & oils
Firestone D (2013j) Method Tb 2-64: moisture by Karl fisher method in industrial oil derivatives. Method Ja 2b-87: moisture by Karl Fischer method in lecithin
Firestone D (2013k) Method Cc 17-95: soap in oil
Firestone D (2013l) Method Ca 12a-02: colorimetric determination of phosphorus in fats and oils
Firestone D (2013m) Methods Cc 13b-45, color: Lovibond (Wesson), and cc 13e-92, color: lovibond (ISO standard)
Firestone D (2013n) Methods Td 1a-64, color: gardner (industrial oils) and Ja 9-87, color: gardner (lecithin)
Firestone D (2013o) Method Cc 13j-97, color (automated method)
Firestone D (2013p) Method Cc 13a-43, color (FAC)
Firestone D (2013q) Method Cc 13c-50, color (spectrophotometric method for oils)
Firestone D (2013r) Method Cc 7-25 refractive index (fats and oils)
Firestone D (2013s) Method Cc1-25, melting point: capillary tube method
Firestone D (2013t) Method Cc 3-25, slip melting point (AOCS Standard); method Cc 3b-92: slip melting point (ISO method)
Firestone D (2013u) Method Cc 18-80, mettler dropping point
Firestone D (2013v) Methods Ja 11-7, viscosity: lecithin (bubble time method); and Da 26-42, viscosity: transparent liquids (bubble time method)
Firestone D (2013w) Method Ja 10-87, viscosity: lecithin (Brookfield)
Firestone D (2013x) Method Cc 10a-25 specific gravity of liquid oils and fats
Firestone D (2013y) Method Cc 10b-25: specific gravity of solid fats and waxes
Firestone D (2013z) Method Ce 1-62: fatty acid composition by GLC of methyl esters; method Cd 28-10: glyceryl fatty acid esters in edible oil
Firestone D (2013aa) Method Cd 11b-91: mono- and diglycerides by capillary GLC
Firestone D (2013ab) Method Cd 11d-96: Mono- and diglycerides by HPLC – ELSD
Firestone D (2013ac) Method Ja 7b-91: phospholipids in lecithin concentrates by HPLC; Method Ja 7c-07: phospholipids in lecithin concentrates by HPLC-ELSD
Firestone D (2013ad) Method Ca 14b-96: free glycerin by HPLC-ELSD
Firestone D (2013ae) Method Cd 25a-00: heat exchange fluid by HPLC

Firestone D (2013af) Method Ca 12a-02: phosphorus in oils (ISO Method)
Firestone D (2013ag) Method Cd 1e-01: iodine value by NIR
Firestone D (2013ah) Method Ca 18-91: lead in oils – graphite furnace
Firestone D (2013ai) Methods 15–75: iron, copper, and chromium by AAS; Ca 18-79: iron, copper, and chromium in oils by AAS - graphite furnace
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Chapter 4
Emulsifier-Carbohydrate Interactions

Lynn B. Deffenbaugh

Surfactants include a range of compounds such as emulsifiers, detergents and polar lipids. Because of the amphiphilic nature of food emulsifiers, they interact with other polar and nonpolar ingredients commonly present in food matrices (Faergemand and Krog 2005). Emulsifier interactions with water, carbohydrates, proteins, fats, oils, and flavors have been studied extensively. These interactions may result in beneficial effects, such as retardation of staling in bread, or adverse effects, such as distortion of a flavor profile. Several mechanisms may be responsible for producing interactive effects: (1) Competition of emulsifiers and ingredients at polar/non-polar interfaces, (2) Competition for available water, (3) Solubility of ingredients in emulsifiers, (4) Electrostatic interactions between charged species, (5) Nonpolar interactions, or (6) Physical or packing interactions, such as entanglement or crystal packing. Since more than one mechanism may be operational in a given food system, explanation of ingredient interactions is often difficult to obtain with a high degree of certainty.

Carbohydrates are ubiquitous in food products. Nutritionally, they serve as sources for rapidly available energy. They also contribute to sensory properties, such as sweetness and texture. Carbohydrates range from low molecular weight simple saccharides to highly complex structures, such as starches and hydrocolloids (Belitz et al. 2004a). Their interactions with food surfactants are extremely important in many foods, such as bakery products. Recently, the epidemics of obesity and Type II Diabetes have stimulated interest in reformulation of foods toward lower sugar content and higher levels of carbohydrates with lower glycemic indices (Warshaw and Kukami 2004). Starch and starchy foods vary in their digestibility and the rate and the duration of glycemic response (Singh et al. 2010). Emulsifier carbohydrate interactions may be different and play different roles in the physical and sensory properties in formulations developed with lower glycemic index carbohydrates instead of sugars.

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This chapter will discuss carbohydrate classes where interactions have been thoroughly studied, but will also point out where not enough is known.

4.1 Surfactant Interactions with Starch

One of the most widely studied types of food surfactant interactions are those with starch. Surfactants can modify starch functional properties due to the ability of some of these amphiphilic compounds to interact with hydrated starch molecules.

Starches have two types of molecules: amylose and amylopectin. Amylose has a linear chain structure, whereas amylopectin has a number of branches. The ratio of amylose to amylopectin depends on the vegetable source (Mitolo 2005). Most normal starches have 20–30% amylose and the balance as amylopectin. So-called "waxy" starches come from natural plant variants with a very high (>85%) amylopectin content in the starch granules, and amylo-starch varieties have a relatively high (>40%) amylose content. Starch molecules are arranged in successive layers into discrete particles or granules that are very distinct between different botanical sources (Greenwood 1976). For wheat starch, the starch properties further depend on fractionation from milling processes (Tang et al. 2005).

The types of organic compounds that can complex with starch include alcohols, aldehydes, terpenes, lactones and lipids as well as surfactants with an aliphatic ligand moiety (Rutschmann and Solms 1990b). Emulsifiers are one group of ligand–containing surfactants that can form starch inclusion complexes. Examples of starch complexing emulsifiers are monoacylglycerols and sodium stearoyl-2-lactylate (SSL). Commonly used food emulsifiers and natural emulsifiers, such as phospholipids, have been used in many investigations on starch complexing behavior. In addition, surfactants, such as sodium dodecyl sulphate (SDS) and CTAB, and pure fatty acids have been used in model systems and studies aimed at elucidation of interaction mechanisms.

Linear left-handed helices of starch form inclusion (or clathrate) compounds with single-tailed surfactants (Carlson et al. 1979). Starch complexing agents have a lipophilic component with a diameter of approximately 4.5–6 Å that will fit into the starch helix (Rutschmann and Solms 1990a). In addition to lipophilic organic compounds, iodine (as I_3) forms inclusion complexes with starch. Complexing agents compete for available space in the starch helix and readily undergo reversible interchange (Schoch and Williams 1944; Mikus et al. 1946). This phenomenon allows starch to be used as an indicator in the titrimetric determination of iodine. Some flavor compounds with an aliphatic moiety can also form complexes with amylose, and this may change a food's flavor or aroma profile (Maier et al. 1987; Schmidt and Maier 1987; Rutschmann and Solms 1990a; Lopes de Silva et al. 2002; Preininger 2005; Ferry et al. 2006; Tietz 2007).

Raw starch granules are insoluble, and uncooked starch is not available to interact with other food ingredients. Cooking is the most common processing method by which functional properties of starch are manifested. Starch molecules that are

solubilized during the cooking process can interact with agents such as lipids and emulsifiers to form complexes. As starch is solubilized during cooking, amylose leaches from starch granules. In the presence of a complexing agent, the amylose converts to a single helix that can complex with a ligand (Carlson et al. 1979). Fatty acid chains, such as those found as a moiety in many emulsifiers, are lipophilic and are attracted to the dipole-induced, hydrogen-lined interior of the helix (Krog 1971). Dipole moments also stabilize the complex by affecting a lipophilic solvation within the starch chain core. The conversion to this configuration is driven by energy minimization principles (Neszmelyi et al. 1987) and a tendency of amylose to minimize its interactions with water (Heinemann et al. 2001). Likewise, participation of a surfactant in the complex will be driven by whether conditions make it energetically more favorable to bind to the starch or to remain in solution (Lundqvist et al. 2002b).

In an inclusion complex, the starch helix can have six to eight glucosyl units per turn (Valletta et al. 1964) and the helix may have two to three turns for each ligand molecule, depending on the size and shape of the guest molecule (Godet et al. 1993). Larger or less linear lipids may induce a larger helix in a complex (Putseys et al. 2010). The long linear structure of amylose allows it to easily adopt the helix formation needed for an inclusion complex. The branches on amylopectin, however, interrupt helix formation. The average length of amylopectin branches is 20–26 glucose residues, which is long enough to partially participate in complexes with complexing agents (Eliasson 1994). It is now known that the outer branches of amylopectin are long enough to bind with surfactants (Eliasson and Ljunger 1988; Gudmundsson and Eliasson 1990; Eliasson 1994; Lundqvist et al. 2002a, b). Because the outer branch amylopectin chains are shorter than amylose, surfactant binding with amylopectin shows Langmuir behavior (low cooperativity) whereas surfactant binding with amylose is known to be highly cooperative (Lundqvist et al. 2002a, b). Amylopectin complexes are more soluble in aqueous systems than amylose complexes, and saturated fatty acids have long been used to selectively precipitate amylose from solution (Schoch and Williams 1944). The relative solubility of amylose and amylopectin complexes can also vary with various surfactants (Kim and Robinson 1979).

When starch/surfactant complexes are unordered and amorphous, they are called Type I amylose inclusion complexes (Putseys et al. 2010). The complexes can organize into lamellar mesophases, which are perpendicular to the helices (Raphaelides and Karkalas 1988). These organized lamellae are called Type II amylose inclusion complexes when they aggregate into crystallized structures. Unit cell packing and the configuration of the Type II lamellar crystallites will be vary depending on the size and nature of the complexing ligand (Putseys et al. 2010). The crystallized structures of amylose inclusion complexes can be identified by a V-pattern in X-ray diffraction (Szezodrak and Pomeranz 1992).

Emulsifiers are added to starch-containing foods for a variety of practical reasons. For example, starch/surfactant complexes retard the firming (staling) of bread, prevent stickiness and promote rehydration in instant potato products, change rheological properties of starch-containing products, and control the texture and stickiness of extruded foods. Complexes between monoacylglycerol, a common food emulsifier,

and amylopectin were shown to be weaker than complexes between monoacylglycerol and amylose (Lagendijk and Pennings 1970; Hahn and Hood 1987; Twillman and White 1988). Nevertheless, emulsifier complexes with amylopectin are of great practical relevance; amylopectin retrogradation is partially responsible for bread staling and emulsifier interactions with amylopectin, albeit relatively weak, have a beneficial impact on bread staling. Formation of starch complexes has also been proposed as a means to protect susceptible aliphatic ligands, such as fish oil, from oxidation (Lalush et al. 2005), as well as a vehicle for controlled lipid release from pre-formed amylose-lipid complexes (Gelders et al. 2006).

Chemical modification of starch is done to modify starch functional properties, but will also affect starch complexing ability. The degree of substitution in a chemically modified starch will affect complexation capacity, and while a low degree of substitution may have minimal effect, higher levels of modification will measurably affect complexing ability (Kim et al. 1992). For example, flavor molecules that complex with un-modified starch were found to dramatically reduce Iodine Binding Capacity (IBC), whereas those same flavors had limited effect on IBC of a chemically modified starch (Tietz 2007). This behavior indicates that the chemical modification of the starch may restrict the formation of extended helical segments that can complex with ligands.

This chapter discusses the ability of surfactants to interact with carbohydrates, especially via inclusion complexes with starch, and focuses on surfactants and emulsifiers intentionally added to modify the properties of starch due to these interactions.

4.2 Effect of Surfactants on Starch Properties

4.2.1 General

Native starches contain small amounts of lipids and some of the native starch exists in situ as starch lipid complexes which affect rheological properties of the starch (Morrison et al. 1993). Fats and oils added to foods contain small amounts of surfactants, which will also affect start properties of starch-containing food. For example, soybean oil contains low levels of lecithin and mono/di-acylglycerols. These native surfactants have functionality as evidenced by a measureable change in surface tension when they are removed by adsorption on Florisil® silica based adsorbents (Gaonkar 1989). The low inclusion rate of these native constituents may be treated as a constant by product developers, providing the concentrations do not vary significantly from lot to lot of the host raw material. Surfactants that are deliberately added are likely to be at a higher and more consistent inclusion rate than the native sources. As such, these exogenously added emulsifiers will exert a greater effect and may be used to deliberately control properties of starches in food formulations.

A variety of experimental methods are used to study starch-lipid complexes, ranging from methods that probe molecular interactions (e.g., X-Ray diffraction and

Iodine Binding Capacity, microscopic properties (e.g., Transmission Electron Microscopy and Confocal laser Scanning Microscopy) to macroscopic behavior (e.g., rheology). Likewise, some methods are empirical (e.g., pasting behavior) and others are direct. The extensive body of research surrounding emulsifier-starch interactions speaks to the complexity of these systems, and sometime conflicting results observed with the range of methods used to study them.

4.2.2 Iodine Binding Capacity

The Iodine Binding Capacity (IBC) is a spectrophotometric method based on the ability of iodine to form an inclusion complex with starch by a mechanism that is similar to formation of a starch/surfactant complex. The method can be used to measure amylose content in starch containing materials. IBC also differentiates amylose from amylopectin, since it forms blue complexes with amylose and a red-purple complex with amylopectin.

Exogenously added surfactants reduce the IBC of normal (non-waxy) starches (Bourne et al. 1960; Osman et al. 1961; Krog and Nybo-Jensen 1970; Krog 1971; Kim and Robinson 1979; Ghiasi et al. 1982a; Moorthy 1985; Deffenbaugh 1990; Conde-Petit and Escher 1992). This effect is due to a reversible exchange between the alkyl chain of the fatty acid moiety and I_3 within the amylose helix. Little or no reduction of IBC has been observed for waxy, high-amylopectin starches (Bourne et al. 1960; Krog and Nybo-Jensen 1970; Krog 1971; Deffenbaugh 1990). This is illustrated in Fig. 4.1, which shows the effect of a sucrose ester emulsifier on Iodine Binding capacity for normal, waxy and high amylose starches.

IBC was reduced for Hylon® VII (high amylose) and normal (maize, potato, tapioca and wheat) starches in the presence of increasing levels of a sucrose ester emulsifier, whereas a change in the IBC of a waxy maize starch was not detected, partially because the IBC of waxy maize starch was so low initially. Even when surfactants have a significant impact on the properties of waxy starches measured by other methods, IBC values for waxy starches are low and the impact of emulsifier interactions are difficult to detect.

IBC provides a general, indirect indication of performance, namely performance related to amylose content. However, IBC may not be sensitive enough to be useful in selection or optimizing emulsifier performance for a specific matrix, especially with high amylopectin starch matrices.

4.2.3 Starch Gelatinization and Pasting

Starches and starch-containing ingredients are largely responsible for the texture of many food products. The role of starch is especially important in fat-reduced or fat-free products, as the starch networks are often used to immobilize free water and prevent syneresis.

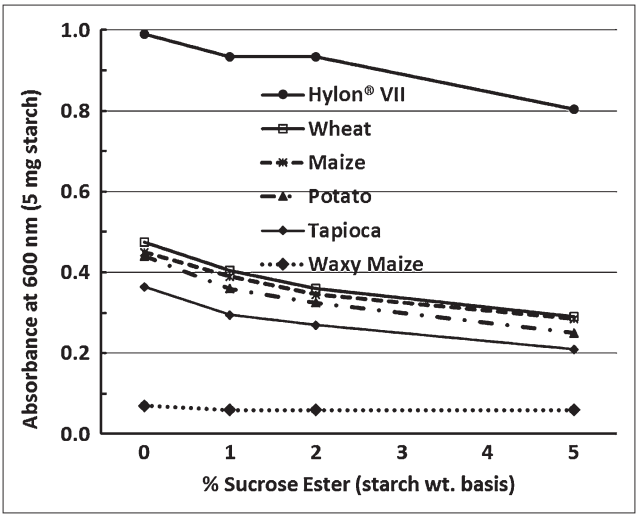

Fig. 4.1 Iodine Binding Capacity of starches measured in the presence of a sucrose ester emulsifier. (From Deffenbaugh 1990)

The functional properties of starch are attained when the starch is cooked, and at least some level of starch gelatinization is generally required. Gelatinization is a process in which the crystalline structure of the starch granule is irreversibly lost. The process is a first-order, water-mediated dissociation of the crystalline regions in the starch granule (Donovan 1979; Zobel 1984). Maximum swelling and solubilization occur in the presence of excess water (a ratio of >5× water to dry starch), which is common in applications such as puddings, sauces, and gravies. Incomplete starch hydration occurs in lower-moisture products, such as baked or extruded products. Extremely high viscosities can be reached in low-moisture systems.

When starches are heated in the presence of water, the starch granules absorb water and swell. Water uptake by starch granules is driven by differences in osmotic pressure (Ito et al. 2004). Differences in osmotic pressure and water ingression concurrently cause linear amylose to leach from the swelling granule (Hernandez-Hernandez et al. 2011). Amylose that has leached from the granule will initially be in a random coil in the surrounding solution. The resulting composition is a mixture of swollen granules, granule fragments, and colloidal starch particles (Olkku and Rha 1978).

The Pasting phase overlaps with and is a continuation of gelatinization. During Pasting, the viscosity of the mixture increases dramatically. However, the swollen starch granules in the paste are very fragile and begin to disintegrate, especially when shear force is applied, such as via mixing. As granule disintegration proceeds, viscosity decreases rapidly. The so-called "Peak Viscosity" is the highest viscosity measured between the competing events of granule swelling and disintegration.

Single, soluble amylose coils are energetically driven to form double helix amylose (Kulp and Ponte 1981). However, once amylose chains have acquired enough

mobility, they are also able to interact with complexing ligands (Evans 1986). As amylose chains gain mobility and move toward the external layers of the granule, they come in contact with emulsifiers in the solution (Hernandez-Hernandez et al. 2011) and form complexes. Induction of starch-lipid complexes in the presence of complexing agents impedes the formation of a double amylose helix with itself (Kulp and Ponte 1981).

Added surfactants tend to stabilize the swollen starch granule. As a result, further granule swelling and starch solubilization are repressed (Strandine et al. 1951; Osman et al. 1961; Krog 1971; Van Lonkhuysen and Blankestijn 1974; Kim and Robinson 1979; Hoover and Hadziyev 1981; Ghiasi et al. 1982a; Moorthy 1985; Eliasson 1986b; Roach and Hoseney 1995a, b; Numfor et al. 1996; Richardson et al. 2003; Toro-Vazquez et al. 2003; Hernandez-Hernandez et al. 2011; Ahmed 2012; Ahmadi-Abhari et al. 2013). This is illustrated in work from Ahmadi-Abhari et al. (2013) who reported that lysophosphatidylcholine (LPC) complexes with wheat starch reduced granule swelling and amylose leakage, which resulted in better preservation of granule integrity throughout cooking. This was visually apparent in light micrographs of iodine stained paste samples as shown in Fig. 4.2. Similar results were seen with stained light micrographs of wheat starch cooked with an emulsifier blend of polyglycerol ester and monoglyceride (Richardson et al. 2003). The emulsifier restricted amylose leakage from wheat starch granules completely up to 90 °C after which the granules suddenly ruptured.

As emulsifiers form complexes with starch, they make a protective layer around starch granules. The layer of complexes at the granule surface retards entry of water into the granules (van Longkuysen and Blankestijn 1974) as well as leaching of soluble amylose out of the granules (Hoover and Hadziyev 1981). The barrier prevents rapid granule hydration, swelling and water diffusion into the starch granule's semi-crystalline region; this increases thermal stability of the granules and allows the granular morphology to remain intact even above the gelatinization temperature of the starch (Hernandez-Hernandez et al. 2011). Some emulsifiers can enter the starch granule and complex internally with amylose (Ghiasi et al. 1982b; Toro-Vazquez et al. 2003; Mira 2006), which further stabilizes the granule in addition to the stabilization provided by the protective layer of complexes on the granule surface.

Figure 4.3 shows that granule swelling and solubility of wheat starch decreased with an increase in complexation between with lysophosphatidylcholine, as measured by the second Differential Scanning Calorimetry transition enthalpy (see Sect. 4.4.2). While the starch granule structure is protected through and above gelatinization temperatures, the protective effect of starch-lipid complex is lost above the dissociation temperature of the complex (Hernandez-Hernandez et al. 2011).

Starch, in its native form, displays birefringence when viewed with a polarized light microscope. Gelatinization and the water mediated loss of order of the crystalline regions in the starch granule, lead to loss of birefringence and disappearance of the characteristic X-ray diffraction pattern (Eliasson 1986a). Starch-complexing emulsifiers have been shown to delay the loss of birefringence (Bourne et al. 1960; Pomeranz et al. 1969; Van Lonkhuysen and Blankestijn 1974; Ghiasi et al. 1982a, b; Ebeler and Walker 1984; Rilsom et al. 1984; Eliasson 1985, 1986a) although not

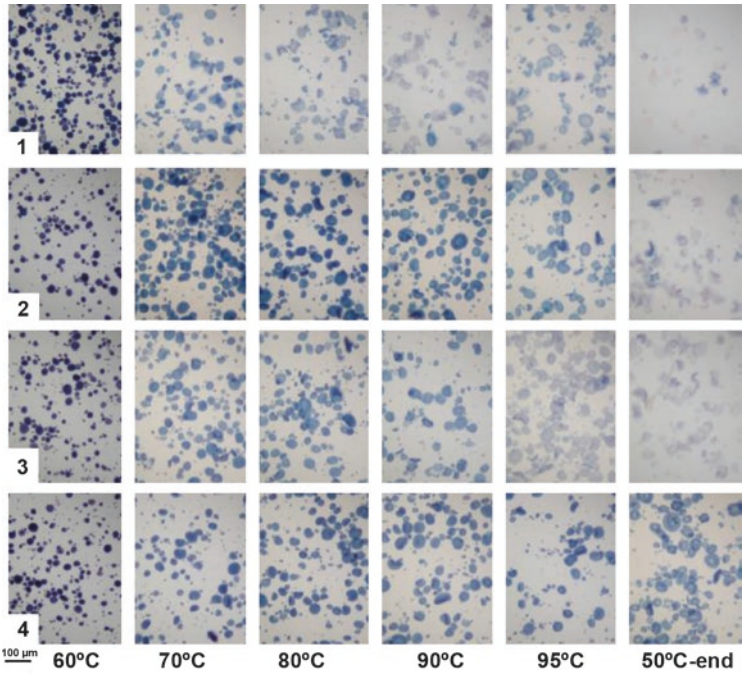

Fig. 4.2 Wheat starch (WS) granules with or without lysophosphatidylcholine (LPC) and stained with iodine under light microscope at temperatures between 60 and 95 °C at end of viscosity profile. Row "1": WS (reference), Row "2": WS + 0.3% LPC, Row "3": WS + 0.5% LPC, Row "4": WS + 1% LPC. (From Ahmadi-Abhari et al. 2013)

with lysophosphatidylcholine addition to wheat starch even though pasting properties were changed (Ahmadi-Abhari et al. 2013).

Pasting temperature and temperature to peak viscosity for normal starches were generally delayed in the presence of long chain food emulsifiers capable of forming inclusion complexes (Favor and Johnston 1947; Pomeranz et al. 1969; Krog 1971, 1973; Eliasson 1983, 1986b; Ebeler and Walker 1984; Rilsom et al. 1984; Evans 1986; Twillman and White 1988; Lin et al. 1990; Xu et al. 1992; Numfor et al. 1996; Mira 2006; Ahmed 2012; Ahmadi-Abhari et al. 2013).

Rapid Visco Analyzer (RVA) pasting data for wheat, corn and potato starches are shown in Table 4.1 (Azizi and Rau 2005). The pasting profiles were affected to different degrees depending on the starch types and the surfactant. These data show that gelatinization temperature increased for wheat starch with some surfactants, whereas gelatinization temperature of corn or potato starch was affected minimally by surfactants. Surfactants also affected Peak Viscosity and Hot Paste Viscosity differently for the different starch types.

Similarly, RVA pasting profiles for maize, potato, tapioca and wheat starches were delayed, to different degrees for each starch type, with the addition of a sucrose ester emulsifier (Deffenbaugh 1990) as shown in Fig. 4.4. The effects of the sucrose

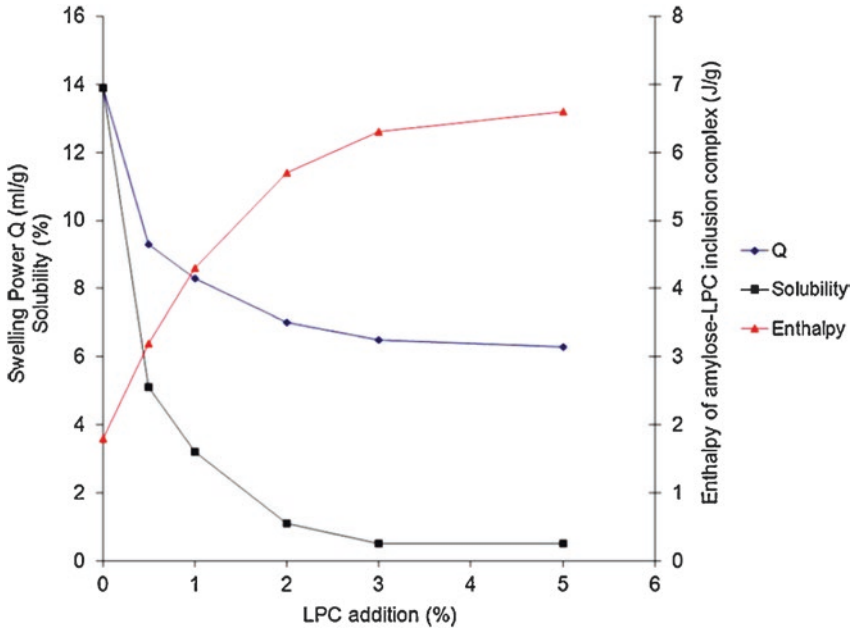

Fig. 4.3 Swelling power and solubility alteration at 90 °C versus enthalpy increase in the second transition (due to amylose-LPC inclusion complexes). Swelling power and solubility are plotted on the left axis against enthalpy. Different points represent different lysophosphatidylcholine (LPC) additions. (From Ahmadi-Abhari et al. 2013)

ester emulsifier on a high amylose, waxy maize starch were modest compared to the effects on normal starches.

The longer the lipid chain length and the higher its concentration, the more the pasting and gelatinization of the starch suspension are delayed (Eliasson 1986a; Raphaelides and Georgiadis 2006). Mira (2006) found that shorter (10 and 12 carbon) alkyl chain emulsifiers induced an earlier pasting while longer (14 and 16 carbon) chain emulsifiers had the opposite effect. Alteration in pasting behavior also depends on the degree of inclusion complex formation, which is influenced by the ratio of ligand to amylose helices (Ahmadi-Abhari et al. 2013).

As a starch paste cools, molecules become less soluble and aggregate (Osman 1967). Gelation occurs when an interconnected network forms upon cooling. A dramatic increase in viscosity generally occurs in normal (non-waxy) starches when a starch paste is cooled to about 70 °C (Osman and Dix 1960), presumably due to this induced gelation. A starch gel formed from a paste is a composite of swollen starch granules embedded in and reinforcing an amylose gel matrix (Ring 1985). Cross-linking of the network increases the viscosity and rheological properties measured as the resistance to an applied external force (Zobel 1984).

Gelation in a starch paste is initiated by rapid precipitation of amylose. Amylose forms gels by entrapping water molecules, swollen starch granules, and granule fragments into a network. Amylopectin tends to crystallize more slowly and requires relatively higher concentrations than amylose to undergo precipitation.

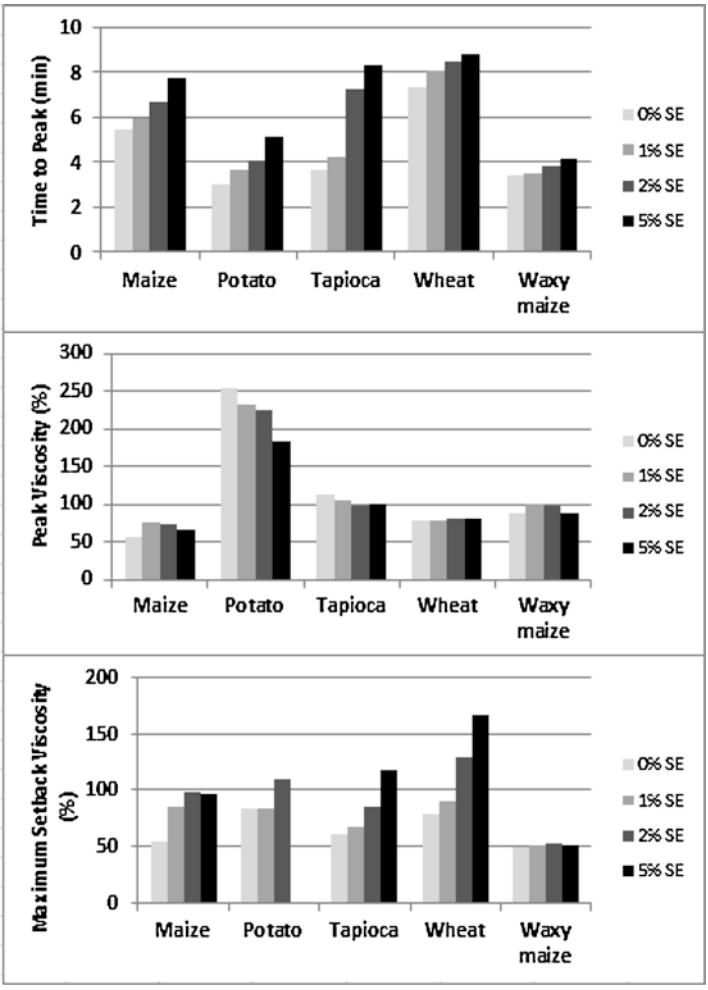

Fig. 4.4 Rapid Visco-Analyzer (RVA) profile parameters for maize, potato, tapioca, wheat and waxy maize starches in the presence of a sucrose ester emulsifier (From Deffenbaugh 1990)

Emulsifiers can also affect the properties of starch gels, and the gel properties have a significant practical impact on cooked food products containing starch. In starch pastes prepared with surfactants, the insoluble starch/emulsifier complex will form a gel (Conde-Petit and Escher 1992). The presence of these complexes accelerates gelation in the first few hours of storage, compared to starch gels made without surfactant (Conde-Petit and Escher 1994). As a result, the setback or cold paste viscosity is often increased (Miles et al. 1985; Evans 1986; Twillman and White 1988; Deffenbaugh 1990; Conde-Petit and Escher 1992; Nuesslil et al. 2000; Heinemann et al. 2001; Richardson et al. 2003; Azizi and Rau 2005).

Cold Paste Viscosity generally increased, sometimes dramatically as seen in Table 4.1, when 0.5% of various surfactants were added to wheat, corn or potato

Table 4.1 Effect of surfactant gels on Rapid Visco Analyzer (RVA) pasting characteristics of wheat, corn and potato starches

Effect of surfactant gels on RVA characteristics of wheat starch

Surfactant gel[a]		Gel temperature (°C)	Peak viscosity (RVU)	Hot paste viscosity (RVU)	Cold paste viscosity (RVU)
Surfactant[b] (0.5%)	Shortening[b] (%)				
Control	–	68.65a	260g	103c	257a
SSL	0	86.30h	249e	100c	398c
	1	92.05i	239d	90bc	430d
	2	94.05j	231cd	90bc	433d
DGMS	0	70.70b	252f	86b	263a
	1	78.06c	227c	91bc	438d
	2	80.05d	201a	101c	506e
GMS	0	80.15d	235cd	80a	258a
	1	80.70e	204a	85b	343b
	2	81.25f	203a	87b	388c
DATEM	0	80.95e	227c	122d	381c
	1	81.35f	210b	122d	382c
	2	82.10g	209b	120d	383c
SEM(±)[c]	–	0.08	2.34	1.21	3.82

Values in the same row followed by different letters differ significantly.
[a] Prepared with surfactant, water and with or without shortening.
[b] Added on starch basis.
[c] Standard error of mean at 39° of freedom.

Effect of surfactant gels on RVA characteristics of corn starch

Surfactant gel[a]		Gel temperature (°C)	Peak viscosity (RVU)	Hot paste viscosity (RVU)	Cold paste viscosity (RVU)
Surfactant[b] (0.5%)	Shortening[b] (%)				
Control	–	73.95a	359e	112d	275a
SSL	0	78.50c	257a	105c	473f
	1	79.60d	257a	106c	616i
	2	79.80d	251a	107c	631i
DGMS	0	74.15a	334d	98bc	296b
	1	74.60b	308cd	94b	509g
	2	75.10bc	298c	90b	552h
GMS	0	74.35ab	336d	104c	326c
	1	74.80b	300c	103c	380e
	2	75.05bc	278b	93b	477f
DATEM	0	74.85b	299c	105c	355d
	1	74.85b	298c	103c	357d
	2	75.00bc	293c	79a	360d
SEM(±)[c]	–	0.07	2.08	1.02	4.13

Values in the same row followed by different letters differ significantly.
[a] Prepared with surfactant, water and with or without shortening.
[b] Added on starch basis.
[c] Standard error of mean at 39° of freedom.

Effect of surfactant gels on RVA characteristics of potato starch

Surfactant gel[a]		Gel temperature (°C)	Peak viscosity (RVU)	Hot paste viscosity (RVU)	Cold paste viscosity (RVU)
Surfactant[b] (0.5%)	Shortening[b] (%)				
Control	–	64.85a	771h	79a	308a
SSL	0	65.80d	496c	93b	490h
	1	66.30g	479b	100bc	459g
	2	66.40h	425a	106c	437f
DGMS	0	65.85de	757g	102bc	345c
	1	65.95e	721de	103bc	334b
	2	66.20f	700d	104bc	332b
GMS	0	66.75d	766gh	107c	395e
	1	65.80d	730e	109c	393e
	2	65.95e	715de	109c	378d
DATEM	0	64.95b	762g	134d	323ab
	1	64.95b	745f	135d	323ab
	2	65.05c	707d	138d	319ab
SEM(±)[c]	–	0.06	4.53	1.04	3.62

Values in the same row followed by different letters differ significantly.
[a] Prepared with surfactant, water and with or without shortening.
[b] Added on starch basis.
[c] Standard error of mean at 39° of freedom.

SSL **sodium stearoyl-2-lactylate,** *DGMS* **distilled glycerol monostearate,** *GMS* **glycerol monostearate,** *DATEM* **diacetyl tartaric acid esters of monoglycerides,** *RVU* **rapid visco analyzer viscosity units. (From Azizi and Rau 2005)**

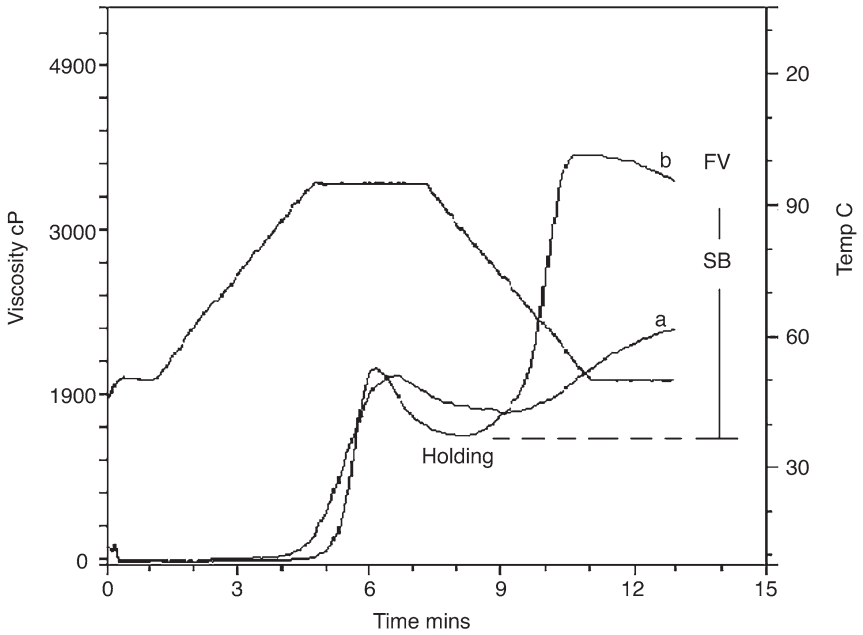

Fig. 4.5 Rapid Visco Analyzer (RVA) pasting profiles of (*a*) wheat starch and (*b*) wheat starch mixed with mono-palmitin obtained using the temperature profile shown. (From Tang and Copeland 2007)

starches (Azizi and Rau 2005) and in Fig. 4.5 where increasing levels of a sucrose ester emulsifier increased setback viscosity for maize, potato, tapioca, and wheat starches (but not waxy maize starch) compared to setback viscosity of the native starches (Deffenbaugh 1990). Similar results were reported for a normal starch by Tang and Copeland (2007) as shown in Fig. 4.5. The significant increase in final viscosity of wheat starch after cooling was found to be due to formation of starch-monopalmitin complexes; a strong linear relationship ($r = 0.967$) existed between increase in Rapid Visco Analyzer final viscosity with increasing levels of monopalmitin and starch complexing index (Tang and Copeland 2007).

As described above, amylose inclusion complexes can induce gelation during cooling. In contrast, some studies have reported decreased cold paste viscosities for starches cooked in the presence of surfactants (Favor and Johnston 1947; Osman and Dix 1960; Krog 1971; Hoover and Hadziyev 1981; Rilsom et al. 1984; Putseys et al. 2010). Putseys et al. (2010, b) explain that in these cases concentrated starch gels formed after cooling may be weakened by starch-emulsifier complexes. This will occur if complexation of amylose restricts gel matrix crystallization (Osman and Dix 1960; Mitchell and Zillman 1951; Hoover and Hadziyev 1981). The reduction of leached amylose during pasting may also contribute to the lower viscosity of the cooled gel (Putseys et al. 2010). This is illustrated in Fig. 4.6 from Putseys et al. (2010).

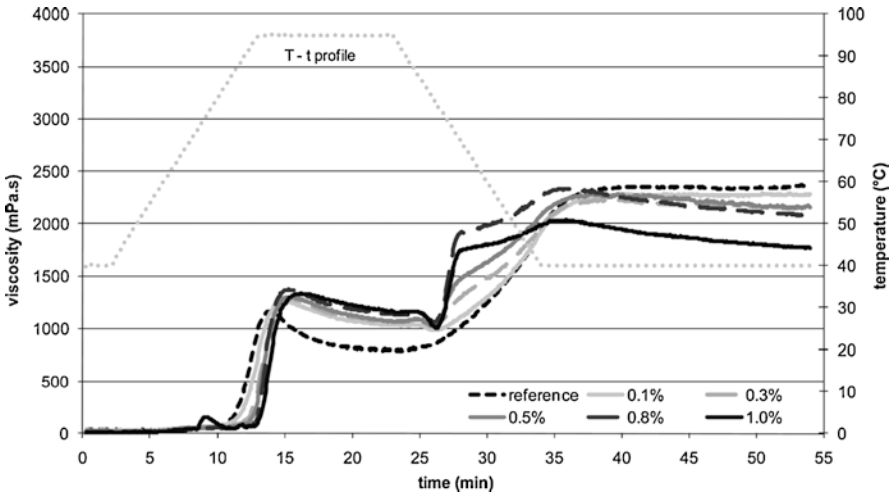

Fig. 4.6 Impact of increasing levels of glycerol monostearate (GMS) on pasting and gelation of a reference starch. (From Putseys et al. 2010)

In the presence of glycerol monostearate (GMS), time to peak viscosity was delayed and viscosity during cooling increased, as expected, versus control starch. However, the final viscosity was lower in the presence of added GMS and may have been due to reduced amylose leaching and the rivalry between amylose-amylose interaction and amylose-surfactant interaction. In light of these competing factors, the impact of ingredient interactions is not always predictable.

Surfactants may be used to induce and control gelation in starch-containing foods (Conde-Petit and Escher 1992) and, as such, use of emulsifiers with starch is a tool for manipulating starch characteristics. When starch is added to a food formulation in order to build viscosity, surfactants, which stabilize the integrity of swollen starch granules, should be selected.

While starch-complexing is one means to induce gelation, some non-complexing surfactants can induce aggregation and formation of a gel network by mechanisms other than starch-complexing (Richardson et al. 2004). In addition, results will vary with raw material sources, their ratios and process conditions.

4.2.4 Starch Retrogradation

Retrogradation is the formation of ordered, partially crystalline regions in a cooled starch paste and the process occurs in two distinct phases. A very rapid gelation of amylose via formation of double helix chains and helix-helix aggregation occurs first, followed by a slower recrystallization of short amylopectin chains (Miles et al. 1985). The first phase of amylose retrogradation occurs so fast that the process may be complete before a starch-containing food product is consumed or distributed.

Complete retrogradation of the starch occurs hours to weeks after pasting and gelation. The second phase of amylopectin retrogradation occurs throughout the shelf life of the product and may cause significant deterioration of texture and flavor attributes (Miles et al. 1985). For example, the increase in firmness and loss of flavor in staled bread are caused by retrogradation of the amylopectin fraction of wheat starch (Schoch and French 1947; Gudmundsson and Eliasson 1990). Control or modification of amylopectin retrogradation by incorporation of surfactants is an important application for surfactants in the food industry. This functionality is due to prevention of side-by-side stacking of starch helices (Miura et al. 1992). Nucleation sites for retrogradation or recrystallization are thereby reduced (Matsunaga and Kainoma 1986).

Interactions between surfactants and amylopectin are weaker than interactions between surfactants and amylose, as described previously. Nonetheless, amylopectin/surfactant interactions have been frequently reported (Bourne et al. 1960; Krog and Nybo-Jensen 1970; Krog 1971; Batres and White 1986; Eliasson 1986b; Eliasson and Ljunger 1988; Evans 1986; Deffenbaugh 1990). Even though these interactions are relatively weak, formation of insoluble complexes between monoacylglycerols and amylopectin have been observed (Batres and White 1986).

While the interactions between amylopectin and lipids are via inclusion complexation, similar to those of amylose and lipids (Eliasson and Ljunger 1988; Gudmundsson and Eliasson 1990), amylose has greater complexing ability. The long amylose chains can form a complex with multiple ligand molecules, so amylose complexation occurs cooperatively once it starts (Lundqvist et al. 2002b); once helix formation begins in order to bind one ligand, formation of helical areas to bind additional ligands is easier. Whereas most of a long amylose chain can complex with ligands, surfactants primarily bind with only the outer branches of amylopectin molecules (Eliasson and Ljunger 1988; Gudmundsson and Eliasson 1990; Lundqvist et al. 2002b). Because the outer branch amylopectin chains are shorter than amylose and can bind only one or few ligands, surfactant binding with amylopectin shows less cooperativity (Langmuir behavior) than with amylose (Lundqvist et al. 2002a, b).

As shown in Fig. 4.4, the effects of a sucrose ester emulsifier on pasting properties, especially setback viscosity, of a high amylopectin waxy maize starch were generally a lower magnitude than the effects on normal (maize, potato, tapioca, and wheat) starches (Deffenbaugh 1990). This is consistent with the relative weakness of the interaction between surfactants and amylopectin compared to amylose.

Differential scanning calorimetry and X-ray diffraction detected the interaction of monoacylglycerols and other surfactants with amylopectin (Gudmundsson and Eliasson 1990). These results were correlated with a reduction of amylopectin retrogradation, a direct result of the interaction between amylopectin and a surfactant. When amylose and amylopectin are present together, surfactants will preferentially complex with the amylose before amylopectin. As a result, the amylose cannot cocrystallize with the amylopectin, resulting in an indirect effect of surfactant on amylopectin.

4.2.5 Enzymolysis of Starch

Starch complex formation with surfactants reduces the rate of in vitro enzymolysis (Ghiasi et al. 1982a; Holm et al. 1983; Eliasson and Krog 1985; Deffenbaugh 1990; Takase et al. 1994; Cui and Oates 1999; Siswoyo and Morita 2003; Gelders et al. 2005; Ahmadi-Abhari et al. 2013). In the case of glucoamylase, an enzyme which cleaves successive glucose units starting at the non-reducing end of a starch chain, complexes formed between sucrose ester emulsifiers and starch were shown to not affect the enzymolysis kinetics of the glucoaylamylase (Deffenbaugh 1990). In vitro digestion measurements demonstrated that complexing wheat starch with 5% LPC leads to a 22% decrease in rate of reducing sugar release by α-amylase (Ahmadi-Abhari et al. 2013). Increasing amylose and lipid chain length increases the enzyme resistance of amylose-lipid complexes (Gelders et al. 2005). The effect of starch complex formation on enzymolysis may be due to reduced solubility of the complexes and steric hindrance, since the surfactants occupy positions between starch helices.

Studies show that starch/surfactant complexes also modify in vivo hydrolysis and digestion of starch. An in vivo study in rats indicated that while overall digestibility of starch/surfactant complexes was similar to the starch control, the complexes amylose was hydrolyzed and absorbed slower (Holm et al. 1983). Cornstarch complexed with a monoacyglycerol was digested less rapidly and lowered blood insulin response in rats; lipogenesis of adipose tissue and liver were reduced (Takase et al. 1994). Mean apparent digestibility of carbohydrates in ileal-cannulated dogs was reduced from 89% for control starch to 76% for V-complex starch (Murray et al. 1998). The authors suggested that replacement of traditional carbohydrates with V-complex starch may be beneficial for diabetic patients because of the decreased digestibility and subsequent glucose absorption rate. Because amylose-surfactant complexes are less easily degraded by enzymes, they may be regarded as part of the resistant starch fraction (Cui and Oates 1999). It has been proposed that the impact of surfactant/starch interactions on carbohydrate digestibility can be used as a tool to modify nutritional impact although it is not known yet if these modifications can be made without significantly affecting the structural functions that the starch provides in foods (Ahmadi-Abhari et al. 2013).

4.3 Factors Affecting Starch/Surfactant Interaction

4.3.1 Surfactant Properties

A wide range of emulsifiers are used in food products, and their functional benefits in various applications are based on interactions with other molecules and modification of the behavior of the other ingredients. The fundamental function of common food emulsifiers is shown in Table 4.2 according to O'Brien (2004). Each emulsifier

Table 4.2 Common emulsifier functionality according to application

Emulsifier type	Emulsifier function				
	Starch complexing	Emulsion stability	Dough conditioner	Crystal modifier	Aeration
Mono- and diglycerides					
– Hard or saturated	1	2	4	3	1
– Soft or unsaturated	3	2	4	3	2
Propylene glycol esters					
– Propylene glycol mono- and diesters	2	5	5	1	1
	1	3	4	1	1
– Propylene glycol mono- and diesters and mono- and diglycerides					
Sorbitan esters					
– Sorbitan monostearate	5	2	5	1	3
Polyoxyethylene sorbitan esters					
– Polysorbate 60	2	3	1	3	1
– Polysorbate 65	3	3	3	3	2
Polyglycerol esters					
– Triglycerol monostearate	3	3	3	3	1
– Hexaglycerol distearate	3	1	3	1	2
Lactylated esters					
– Stearoyl-2-lactylate (SSL)	2	1	1	5	2
Lecithin					
– Standard fluid grade	3	3	3	1	4

Emulsifier Functionality Evaluation: 1 = excellent; 2 = good; 3 = slight; 4 = poor; 5 = none (O'Brien 2004)

tends to be best suited for one or two functions, and no single emulsifier exhibits a high level of all of the functional characteristics. Note that the best starch complexing emulsifiers are mono- and diglycerides and propylene glycol mono- and diesters and mono-and di-glycerides. These starch complexing emulsifiers are glycerol or propylene glycol esters that have an alkyl chain that forms the inclusion complex with single helices of starch as discussed above. Sodium stearoyl 2-lactylate (SSL) is an ionic surfactant, a stearic acid ester of lactic acid that is partially neutralized with a base, with a relatively high starch-complexing capability.

Emulsifier functionality defines its application and benefit. Emulsifiers that can complex amylose and amylopectin and reduce retrogradation reduce initial bread crumb firmness (Lagendijk and Pennings 1970; Stampfli and Nersten 1995; Sawa et al. 2009). Blends of glycerol monostearate (GMS) and sodium stearoyl-2-lactylate (SSL) added to taro paste, reduced the toughness, cohesiveness and elastic quality of the cooked paste gels during storage versus control (Raksaphol 2009). Likewise, in extruded rice vermicelli, addition of monoacylglycerides reduced final product stickiness (Charutigon et al. 2008).

However, some surfactants do not form complexes with starch. Sodium dodecyl sulfate (SDS) is an ionic surfactant that is a salt of a strong acid and a strong base, but does not complex with amylose. SDS has a strong destabilizing effect on starch granules, possibly because of its strong negative charge, detergent power or high potential to form micelles (Moorthy 1985; Eliasson 1986b). Destabilization is manifested by a rapid swelling and viscosity increase, followed by granule disruption and viscosity decrease.

Within the starch complexing emulsifiers, lipid chain length, degree of fatty acid chain unsaturation, identity of polar head group and other factors further define starch complexing ability and resulting functional performance. In addition, other factors such as wetting or water transport into the granule may be at play as well (Mira 2006).

Single-tailed surfactants with saturated alkyl chains are well suited for complexation with starch. Solubility of the lipid or surfactant determines the equilibrium concentrations of the complex and the lipid in solution. The more soluble the lipid complexing agent, the greater proportion will be present in the aqueous phase. Lipids with 10 or less carbon atoms appear to not be able to induce formation of crystalline starch complexes because they are too soluble to be retained in the hydrophobic helix cavity (Karkalas and Raphaelides 1986; Tufvesson et al. 2003b). On the other hand, longer fatty acids are less soluble than monoacylclycerols with comparable fatty acid chain lengths. Therefore, a fatty acid has higher complexing ability than its corresponding monacylglycerol (Hahn and Hood 1987; Tufvesson et al. 2003a, b). Triacylglycerols do not form complexes with starch (Tang and Copeland 2007); lipids with low water solubility have a tendency to self-associate rather than form complexes. Differential solubility at higher processing temperatures and storage temperatures should also be considered.

When complexation occurs, binding increases as the alkyl chain length increases (Gray and Schoch 1962; Hahn and Hood 1987), as shown by an increase in the energy required to dissociate amylose-lipid complexes as the length of the aliphatic chain of the lipid increases (Stute and Konieczny-Janda 1983; Eliasson and Krog 1985; Tufvesson et al. 2003a, b; Gelders et al. 2005). Maximum binding was generally with 14–18 carbon fatty acid chains. The effect of fatty acid chain length in glycerophosphatidyl-choline on ability to suppress amylose leaching reduced from 14 to 16 to 18 carbons in parallel to the ability to be accommodated in the amylose helix (Siswoyo and Morita 2003).

Increased unsaturation in the fatty acid chain reduces the ability of the lipid to form amylose inclusion complexes and stability of resulting complexes (Lagendijk and Pennings 1970; Krog 1971; Stute and Konieczny-Janda 1983; Eliasson and Krog 1985; Hahn and Hood 1987; Raphaelides and Karkalas 1988). This is especially true for the cis-unsaturated lipids, where the 30° angle of the 9, 10 cis (Z) double bond in the fatty acid chain reduces rotational flexibility and produces steric hindrance to insertion into the helix (Lagendijk and Pennings 1970).

The polar head group on a ligand molecule will be located outside of the two or three turns of the starch helix for each ligand due to steric and/or electrostatic repulsions (Godet et al. 1993). A bulky polar group, such as with propylene glycol esters

of fatty acids, poses an additional steric barrier to complex formation (Krog 1971; Hahn and Hood 1987). An ionic charge on the polar head of a surfactant will tend to increase the water solubility of the complex that is formed with amylose (Mira 2006).

The amylose-complexing ability of surfactants containing alkyl chains is affected by their phase behavior (Larsson 1980). The most effective complexing surfactants have a high degree of freedom in the aqueous phase and exhibit lyotropic mesomorphism. Micelles and vesicles (liposomes) are the mesophases that are the best sources of surfactant monomers for complex formation. For example, monoacylglycerols exist as micelles in an aqueous environment at low temperatures (<50 °C), and attach to the starch granule surface by adsorption (Van Lonkhuysen and Blankestijn 1974). As the temperature is increased to >80 °C, the starch granules swell, and the alkyl chains of the monoacylglycerol penetrate the starch helix. Other mesophases (lamellar, hexagonal, cubic) are less effective (Rilsom et al. 1984; Eliasson 1986a). Richardson et al. (2003) showed that physical state of emulsifiers affected complex formation; α-gel monoacylglycerols (MG) restricted wheat starch granule swelling at 50 °C whereas β-crystal MG did not.

4.3.2 Starch Type and Source

Starch is a high molecular weight biopolymer with a molecular structure that varies according to its biological source. As previously discussed, the major variation is the relative proportion of amylose and amylopectin (Mitolo 2005). Structural differences in the granules also affect the properties of surfactant/starch complexes.

Some traditional methods of analysis, such as iodine binding capacity and glucoamylase digestions, are not sufficiently sensitive to measure the subtle differences due to differences in starch type. Other methods, such as measurement of viscoelastic properties (Eliasson 1986b) and viscosity (Deffenbaugh 1990) are capable of differentiating the responses of different starch types in the presence of surfactant. For example, glycerol monostearate (GMS) restricted swelling of potato starch granules to a greater extent than it did for maize or wheat starch granules (Eliasson 1986b).

Viscosity parameters for various starches in the presence of sucrose ester surfactants are shown in Fig. 4.4 (Deffenbaugh 1990). The time to peak viscosity changed more for tapioca than for maize, wheat, and potato starches. The surfactant affected setback viscosity most in the wheat starch. Potato and tapioca granules were stabilized by complex formation so that swelling and disintegration were more gradual. Starch-complexing surfactants also stabilize the pasting viscosity of tapioca starch (Moorthy 1985). Viscosity profiles are convenient for studying complex properties in food systems and are also directly relevant to food product specifications.

4.3.3 Process Conditions

Amylose-lipid complexes are formed under a range of processing conditions for starch-based systems including extrusion (Bhatnagar and Hanna 1994; Charutigon et al. 2008), parboiling (Lamberts et al. 2009) and bread making (Krog and Nybo-Jensen 1970; Stampfli and Nersten 1995).

Temperature affects the stability of starch/surfactant complexes and consequently affects their functionality in food systems. Iodine and fatty acid binding capacities of amylose decrease with increasing temperature (Banks and Greenwood 1975; Hahn and Hood 1987). The starch helix becomes more disorganized which reduces its ability to include complexing agents. Increasing temperature may also increase the solubility and mobility of complexing agents in the aqueous phase.

Binding of some fatty acids by amylose is affected by pH via protonation and deprotonation of the carboxyl group (Hahn and Hood 1987). Palmitic (C-16) and stearic (C-18) acids form dimers below their pK_a values (4.7–5.0) by hydrogen bonding between their protonated carboxyl groups. Twinning of their alkyl chains makes them too bulky to fit into the amylose helix. Above their pK_a, the carboxyl groups are deprotonated, and the dimer dissociates due to electrostatic repulsion; single fatty acid chains are able to form amylose complexes. The pH does not affect the binding of lower fatty acids, such as myristic (C-14) or lauric (C-12) that do not form dimers. Nonionic surfactants, such as monoacylglycerols, are not affected because the carboxyl group is bonded in an ester linkage and is unavailable for protonation and deprotonation.

Content of the ligand or the ratio of the emulsifier to starch will affect complexation (Deffenbaugh 1990; Richardson et al. 2003; Putseys et al. 2010). As discussed earlier, amylose preferentially forms complexes with surfactants before amylopectin (Eliasson and Ljunger 1988; Gudmundsson and Eliasson 1990), but as concentration of the complexing ligand increases amylopectin will also participate in complex formation (Rutschmann and Solms 1990b).

Maximum complex formation, indicated by effect on final pasting viscosity, was found to occur at a different concentration for different lipids and monopalmitin (Tang and Copeland 2007). With saturated fatty acids, complexation increased with concentration of the complexing agent up to a point after which complexation decreased due to reduced solubility and self-association of the agent in an aqueous solution. This was thought to be related to the water solubility and critical micelle concentration (CMC), the concentration at which micellar aggregation of the lipid occurs. While Mira (2006) reported that the initial Pasting Temperature of wheat starch or flour was not affected by emulsifier CMC, perhaps impact of emulsifier complexing ability was not fully apparent at that early stage in pasting.

Starch gelatinization is a water mediated process, and water content also affects lipid-starch complexation (Eliasson 1980; Eliasson et al. 1981). With excess (>80%) moisture, there was no shift in the onset temperature of the starch gelatinization

endotherm for wheat starch combined with lysophosphatidylcholine (Ahmadi-Abhari et al. 2013). Similar results (no shift in onset temperature) were seen when sucrose esters were heated with maize, potato, tapioca and wheat starches at 70% moisture (Deffenbaugh 1990). Water functions as a plasticizer during formation of an amylose-lipid complex (Jovanovich et al. 1992). At lower (36–64%) moistures, amylose—lipid complexation reduced the enthalpy and increased the onset temperature of the first endotherm indicating that complex formation was delayed in the reduced moisture environment.

Process parameters, such as time of addition of an emulsifier, can be varied to produce cooked starches, or cereal grain products, with significantly modified properties (Lund 1984). For example, if monoacylglycerols are added before starch gelatinization occurs, the surfactants penetrate the starch granule, form complexes and reduce granule swelling power; whereas addition of monoacylglycerols after starch gelatinization stabilized the starch granule against rupture and additional amylose solubilization (Van Lonkhuysen and Blankestijn 1974). These observations are corroborated by work from Exarhopoulos and Raphaelides (2012). When a fatty acid was added to amylose prior to heating, higher degrees of crystallinity, due to amylose-fatty acid complex formation, were seen by X-Ray diffraction compared to amylose to which the fatty acid was added after heating. Some surfactants added prior to gelatinization (e.g., polysorbate 60), adsorb to the surface of the starch granule (Kim and Walker 1992). The surface is rendered lipophilic, which retards the migration of water into the granule. The complexing agent must be available to interact during starch cooking to have an effect on starch properties. A complexing agent added to starch systems as a pre-formed starch-lipid complex had minimal effect on retrogradation of a starch system (Gudmundsson 1992).

4.4 Physical Properties of Starch/Surfactant Complexes

The functionality of emulsifiers in starch-containing food systems can be demonstrated by measuring the impact of emulsifiers on other ingredients or food systems. Examples include the data from starch pasting profiles and enzymolysis that were reviewed previously. In addition, directly measuring the physical properties of starch/surfactant complexes has provided valuable insights into their identification, and has broadened the understanding of the functionality of surfactants in starch-containing food systems. Techniques, such as X-ray diffraction, differential scanning calorimetry, nuclear magnetic resonance, and electron spin resonance, rheology and microscopy have proven especially useful. The best understanding comes from using multiple approaches including empirical and direct methods of measurement.

4.4.1 X-Ray Diffraction Patterns

X-ray diffraction was one of the first techniques used to identify starch inclusion complexes (Mikus et al. 1946). The transition of amylose from a coil to a helix configuration of the inclusion complex increases the order of the molecular structure of amylose (Biliaderis and Tonogai 1991). Clathrates (inclusion complexes) of starch are detected when a powder diffractogram displays a "V-pattern." X-ray diffraction has been widely used to detect an inclusion complex when starch has been heated in the presence of a native lipid or a surfactant (Hanna and Lelievre 1975; Hoover and Hadziyev 1981; Ghiasi et al. 1982; Eliasson and Krog 1985; Eliasson 1988; Biliaderis and Galloway 1989; Deffenbaugh 1990; Rutschmann and Solms 1990a; Tietz 2007; Hernandez-Hernandez et al. 2011; Ahmed 2012; Exarhopoulos and Raphaelides 2012). The helical structure of amylose within the complex was also characterized. X-ray diffraction also displayed V-type patterns for complexes formed between amylopectin and surfactants (Gudmundsson and Eliasson 1990). Studies also indicated that "free" amylopectin formed inclusion complexes, while amylopectin in waxy maize starch did not (Evans 1986; Eliasson and Ljunger 1988).

4.4.2 Differential Scanning Calorimetry

Calorimetry is one of the most extensively used techniques to study interaction between starch and surfactants, especially the nature of the inclusion complexes. When a sample is heated or cooked while accurately measuring temperature, the temperature and enthalpy of thermal transitions are detectable by differential scanning calorimetry (DSC). Gelatinization of starch is a water-mediated endothermic transition that reflects the loss of the double-helical order of raw molecules within starch granules. An endotherm corresponding to starch gelatinization is observed when samples containing raw starch are heated in excess water; this endotherm typically occurs at temperatures less than 80 °C. This gelatinization endotherm is not observed in re-scans of DSC sample because the gelatinization process is irreversible. A second endotherm corresponding to the dissociation of the amylose-lipid complex typically occurs between 90 and 115 °C, and this endotherm is observed in re-scans because the formation and dissociation of this complex is reversible.

As discussed previously, empirical measures of starch pasting generally show a delay in granule swelling, amylose leaching and pasting. The direct measure of thermodynamic loss of starch granule crystalline order in the presence of surfactants measured via DSC shows varying results. Some emulsifiers have been found to increase T_0G, the onset temperature for gelatinization, by a few (e.g., 1–4 °C) degrees (Mira 2006). The magnitude of the effect is affected by starch type, the type and concentration of the surfactant and heating rate. Observations vary, however;

Table 4.3 Gelatinization enthalpy of starches with a sucrose ester emulsifier measured by differential scanning calorimetry

	Sucrose ester emulsifier level			
Starch type	0%	1%	2%	5%
Maize	13.44[1]	11.50[2]	10.61[2]	10.66[2]
Potato	16.93[1]	16.64[1]	16.26[1,2]	15.37[2]
Tapioca	18.19[1]	15.28[2]	13.77[1]	11.83[1]
Wheat	10.61[1]	9.58[1,2]	9.33[2]	8.78[2]
Waxy maize	16.90[1]	17.01[1]	16.96[1]	16.83[1]

Superscripts 1 and 2 indicate significant differences ($p < 0.05$) within a starch type
From Deffenbaugh (1990)

T_0G (onset temperature for gelatinization) and T_PG (peak temperature of gelatinization) of wheat starch were reduced by 9.4 °C and 14 °C, respectively, in the presence of 4% sodium stearoyl-2-lactylate (Ahmed 2012). In contrast, T_0G and T_PG were unchanged by lysophosphatidyl choline for wheat starch (Ahmadi-Abhari et al. 2013) or maize starch (Toro-Vazquez et al. 2003); similarly T_0G and T_PG were unchanged for maize, potato, tapioca, what or waxy maize starches in the presence of sucrose esters (Deffenbaugh 1990).

While T_0G and T_PG of starches show little or no change in the presence of surfactants, the enthalpy of the starch gelatinization endotherm is affected significantly for amylose or amylose containing starches. Starch/surfactant complex formation is an exothermic reaction that occurs during gelatinization. It is consistently reported that complex formation reduces the net enthalpy of the starch gelatinization endotherm (Ghiasi et al. 1982b; Eliasson 1988; Deffenbaugh 1990; Siswoyo and Morita 2003; Toro-Vazquez et al. 2003; Ahmadi-Abhari et al. 2013).

Enthalphy data for several starches interacting with a sucrose ester emulsifier are shown in Table 4.3 as illustration (Deffenbaugh 1990). The Enthalpy (ΔH, J/g) for the first endotherm corresponding to starch gelatinization decreased as the level of a sucrose ester emulsifier increased for normal (maize, potato, tapioca and wheat) starches which have approximately 14–20% amylose. No significant change in enthalpy was detected with waxy maize (high amylopectin) starch with the addition of a sucrose ester emulsifier (Deffenbaugh 1990) or long chain food emulsifiers (Mira 2006).

Starch/surfactant complexes are stable enough to not dissociate at the temperatures of starch gelatinization (e.g., less than 80 °C) (Hernandez-Hernandez et al. 2011). However, the complexes do dissociate at higher temperatures (e.g., greater than 90 °C) and, do so reversibly. Multiple DSC scans have been used to confirm the existence of starch/lipid complexes (Hoover and Hadziyev 1981; Eliasson 1988; Staeger et al. 1988; Deffenbaugh 1990; Szezodrak and Pomeranz 1992; Toro-Vazquez et al. 2003; Ahmed 2012; Ahmadi-Abhari et al. 2013). The enthalpy of the second endotherm increases as emulsifier content increased indicating that complex formation increases with increasing ligand content (Deffenbaugh 1990; Toro-Vazquez et al. 2003; Ahmadi-Abhari et al. 2013).

Physical properties of starch/surfactant complexes also depend on conditions during crystallization. Multiple melting endotherms of complexes or shifting of the

endotherm during re-scanning indicates the presence of different crystal polymorphic forms (Kugimiya and Donovan 1981; Bulpin et al. 1982; Paton 1987; Eliasson 1988; Biliaderis and Galloway 1989). Complexation during first heating may be incomplete due to restricted mobility of the amylose chain during the gelatinization process such that the full extent of complexation will not occur until the amylose has leached from the granule (Kugimiya and Donovan 1981; Toro-Vazquez et al. 2003). These steric constraints on the amylose may result in imperfect complex formation during the first heating (Evans 1986). Different polymorphic forms may occur simultaneously within a large crystal, which has folded back on itself (Eliasson 1988). Complexes in folds or on the surface of the crystal have lower melting temperatures than those further inside the crystal. Knowing that the complex formation is imperfect during the initial cooking phase should be considered when designing the process when emulsifier starch complexation is needed.

The relative thermal stability of starch/lipid complexes can be measured using DSC. Evans (1986) reported that the melting temperature of the amylose-lipid complex was a measure of the average stability of a lengthy stretch of amylose interaction with lipid (emulsifier). The second endotherm for amylose/surfactant complexes is distinct and occurs over a short time period; dissociation of the complex corresponding to this second endotherm is cooperative just as formation was (Lundqvist et al. 2002a). In contrast, the second endotherm for amylopectin/surfactant is non-cooperative, diffuse and, while it occurs, the change in enthalpy may be lost in the background noise such that no discernible peak will be observed. Just as there is lack of cooperativity during complex formation for amylopectin, lack of cooperatively during amylopectin complex dissociation has been proposed. Thermal stability and complex-melting enthalpy decrease as the fatty acid chain is interrupted by cis (Z) double bonds where chain length of the fatty acid does not affect complex melting enthalpy (Hoover and Hadziyev 1981; Stute and Konieczny-Janda 1983; Eliasson and Krog 1985; Raphaelides and Karkalas 1988).

Measuring the formation and stability of starch/emulsifier complexes is of interest because it can predict rheological properties during gelatinization of starch systems (Eliasson 1986b). For example, glycerol monostearate (GMS) forms very stable complexes with starch and has very significant effects on starch gelatinization (Hoover and Hadziyev 1981; Eliasson and Krog 1985). In taro paste, sodium stearoyl-2-lactylate showed a larger amylose-emulsifier complex melting endotherm than a similar level of monoacylglycerols (Lai 1998); emulsifiers that formed complexes with amylose decreased paste hardening during storage under refrigeration.

In addition to using emulsifiers to modify starch performance characteristics, potential use of pre-formed amylose/surfactant complexes as an ingredient have been proposed. Examples include temperature controlled release agents for lipids (Gelders et al. 2006) and V-complex starch as a source of reduced Glucose Index carbohydrates for diabetic patients (Murray et al. 1998). In order to use inclusion complexes as an ingredient themselves in such applications, understanding the differences in properties of Type I and Type II starch inclusion complexes will be of interest (Putseys et al. 2010) and techniques such as DSC will be essential.

4.4.3 Infrared Spectroscopy

Infrared spectroscopy is a useful technique to probe the structure of a surfactant inside the amylose helix. Frequencies for the carboxyl (Osman et al. 1961; Batres and White 1986), methyl (Batres and White 1986), and carbonyl (Hahnel et al. 1995) groups have been investigated. The carbonyl group in glycerol monostearate displays a positive shift inside the complex. This is thought to occur because of electron delocalization inside the helix.

4.4.4 Electron Spin Resonance

Stable free radical fatty acid spin probes may be measured using electron spin resonance (ESR). The line shapes in the spectrum are indicative of the environment surrounding the probe. Reduction in the mobility of the spin probe, due to adsorption or inclusion in a viscous medium, is indicated by line broadening. The technique has been used to study the interactions between fatty acids and starch. Labelled fatty acids where shown to form complexes with both amylose and amylopectin (Biliaderis and Vaughan 1987).

The motion of the fatty acid probe was greatly slowed in the presence of wheat, high amylose maize and waxy maize starches (Pearce et al. 1985). Binding was weaker in waxy maize than in other starches. Results were similar at room temperature, and heating to 90 °C and cooling back to room temperature. Binding was thought to occur throughout the granule, since surface adsorption would not account for the amount of probe utilized. The presence of water facilitated binding, presumably by allowing greater penetration into the interior of the granule (Pearce et al. 1985; Nolan et al. 1986). The facilitating role of water was consistent with DSC data showing that water functioned as a plasticizer during formation of an amylose-lipid complex (Jovanovich et al. 1992). Similar results were found for probes binding to maize and waxy maize starches at room temperature (Johnson et al. 1990). Heating and subsequent cooling were found to destabilize the complex. Heating increases overall spin probe binding by increasing the surface area of the granule and the permeability of the starch granule.

4.4.5 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) measures chemical shifts for odd-numbered atoms or their isomers (^{1}H, ^{13}C, ^{17}O, ^{31}P). The chemical environment near the nuclei influences the position and shape of the peak in the spectrum. For example, stereochemistry in a molecule may be determined with the Nuclear Overhouser Effect (NOE). ^{13}C NMR can detect changes in the carbon atoms in starch induced by complex formation with surfactants (Jane et al. 1985; Deffenbaugh 1990).

Downfield shifts were observed for all carbon atoms of starch, which have been converted into an inclusion complex (Jane et al. 1985). However, shifts for C-1 and C-4 were the most pronounced, suggesting a rotation of the C–O bond in the glycosidic linkage. ^{13}C NMR of maize starch in solution displayed a downfield shift of C-1 and C-4 at 55–75 °C in the presence of a complexing agent (Deffenbaugh 1990). At temperatures above 70 °C, no effect was observed. Although the complex was formed during gelatinization, it could not be detected in solution. Waxy maize starch/surfactant complexes could also be detected by ^{13}C NMR. In situ complexes between non-waxy barley amylose and lysophospholipid were confirmed by ^{13}C Cross Polarization/Magic Angle Spinning NMR; complexation was indicated by narrowing of all polysaccharide carbon resonances as well as a profile for mid-chain methylene carbons of the fatty acids that were sterically restrained in V complex amylose (Morrison et al. 1993). ^{13}C NMR investigations of ligands were used to show that the polar groups on emulsifiers will most likely be located outside of the amylose helix cavity (Snape et al. 1998).

Proton (^{1}H) NMR has also been utilized to study complex formation. The signal intensity of the amylose protons was reduced when sodium palmitate was added. This was interpreted as loss of conformational mobility in the helix due to complex formation, which resulted in extreme line broadening (Bulpin et al. 1982). Signal intensity was restored when the system was heated to >90 °C, apparently due to dissociation of the thermally reversible complex. In a study of cycloheptaamylose, signals for H-3 and H-5 were shifted upfield in the presence of lysolecithin (Kim and Hill 1985). Since these protons were directed toward the interior of the helix, they experienced a more hydrophobic environment after complex formation with the lipid. No band shifts were observed for complexes between amylopectin and monoacylglycerols (Batres and White 1986).

Decoupled ^{17}O NMR was used to study the stability of taro pastes toward retrogradation during storage (Lai 1998). Shifts in signals indicated that water, sugar and starch mobility were reduced in the presence of monoacylglycerols and sodium stearoyl-2-lactylate.

4.4.6 Rheological Properties

Rheology is a discipline, which employs mechanical testing to measure the properties of materials under simulated conditions of use. In foods, the tests attempt to discover component interactions, which define the textural attributes, which make foods desirable to consumers (McClements 2004; Chakrabarti 2005). The impact of starch/lipid complexes on rheological properties is often used to manage their functionality in high-starch foods. Important measurements are storage modulus, loss modulus and gel strength.

When starch pastes were prepared with glycerol monostearate (GMS) or sodium stearoyl-2-lactylate (SSL), changes in viscoelastic properties coincided with reduced swelling of the granules (Eliasson 1986b). The granules were less deformable, as indi-

cated by the higher temperatures required to reach peak values for storage modulus (G') and loss modulus (G"). When flavor compounds formed complexes with starch, storage modulus G' increased continuously with decreasing IBC which confirmed that gelation was induced by complex formation (Tietz 2007). Toro-Vazquez et al. (2003) showed that peaks in the phase shift angle, δ, for wheat starch pastes coincided with formation of a three dimensional starch gel network, and that these δ peaks were absent when lysophosphatidyl choline restricted amylose leaching in maize starch.

In concentrated potato and wheat starches, dynamic modulus was higher in the presence of GMS and SSL due to complex formation with amylose (Keetels et al. 1996). Less gel stiffness occurred with these surfactants during storage. Amylopectin potato starch produced soft shear thinning gels in the presence of GMS and calcium stearoyl lactylates (Nuesslil et al. 2000). The Power Law and the Bird-Leider models were used to determine the effects of triacylglycerol and monoacylglycerol additions to starch pastes (Navarro et al. 1996). Modeling has also been used to investigate starch retrogradation (Farhat and Blanshard 2001). Rheological measurements also determined functionality in some challenging bakery products, such as cake batter (Sakivan et al. 2004), microwaveable cakes (Seyhun et al. 2003) and frozen bread doughs (Ribotta et al. 2004). Using a unique approach, it was suggested that pre-formed amylose/lipid complexes may have utility as controlled release ingredient to release lipids during processing as a means to control rheology of a food system (Gelders et al. 2006).

4.4.7 Microstructure of Starch Systems

Observation of the structure in model systems by microscopy techniques can provide information about functionality and interactions (Groves 2005).

On a macroscopic level, interaction between amylose and menthone, a flavor that formed inclusion complexes, increased turbidity in dilute starch dispersions (Tietz 2007). This was due to aggregation of the complexes into a network over storage time. These aggregated starch-flavor complexes were also observable in light micrographs.

The gross structure of a food matrix can be examined by light microscopy. In principle, objects >200 μM are detectable, but this level of resolution is difficult to achieve in practice. Use of stained light micrographs in starch models systems were discussed previously (Richardson et al. 2003; Ahmadi-Abhari et al. 2013) and an example is shown in Fig. 4.2. Titoria et al. (2004) applied this technique to food products, pastilles and yogurts; interactions of surfactants with starch granules were observed by staining the ingredients.

Cross-polarized light highlights those structures which display birefringence; sugar particles show up as white grains while starch granules show up as a chrematistic "Maltese cross." When starch gelatinizes, the Maltese cross disappears. The rate of gelatinization can therefore be measured in model starch gels or high-starch products (Nuesslil et al. 2000; Lamberti et al. 2004; Seetharamass et al. 2004).

Confocal laser scanning microscopy (CLSM) is useful because sectioning of samples treated with fluorescent probes results in a three dimensional image in which specific components are stained by the fluorescent dyes. For example, three

dimensional images of starch based model food system (Tietz 2007) were obtained. CLSM was also used to show that a shorter chain (C12) surfactant penetrated farther into a starch granule during gelatinization than a longer chain (C16) surfactant (Mira 2006) suggesting that the C12 surfactant may have more access to interact with granule components than the C16 surfactant.

If electrons are used instead of light, much greater resolution of the structure can be obtained. In scanning electron microscopy (SEM), the surface of the sample is observed by scattering of electrons. The sample may be pre-fractured to see interior structure. Transmission electron microscopy (TEM), electrons are passed through a thin section of the sample. Interactions of ingredients may be detected by effects on microstructure of models systems and foods (Olsson et al. 2003). TEM, for example showed that fine-stranded amylose gels transformed into thicker strands by surfactants, but became spheres at higher surfactant concentrations (Richardson et al. 2004). SEM micrographs were used to show that loss of maize starch granule structure was delayed by lysophosphatidylcholine (Hernandez-Hernandez et al. 2011); similarly, SEM demonstrated that formation of the network structure needed to form a gel was delayed when lydophosphatidylcholine was added to maize starch (Toro-Vazquez et al. 2003). SEM was also used to observe the impact of glucoamylase digestion on starch granules (Siswoyo and Morita 2003); erosion and holes in the granules were reduced when starch was complexed with monoacyl-sn-glycerophosphatidylcholine.

4.5 Surfactant Interactions with Simple Saccharides

Simple saccharides, such as sucrose, fructose, or lactose occur naturally in foods or are added to obtain some benefit. Sugars contribute sweetness in varying degrees depending on their structural configuration. However, they also function as humectants to retain water and reduce water activity, in order to improve microbial stability. For example, water activity has an effect on cell permeability of Staphylococcus aureus (Vilhelmson and Miller 2002). Other ingredients such as glycerol, propylene glycol, and sorbitol, also function as humectants.

Because there are no lipophilic groups in simple saccharides, these molecules have little or no interfacial activity. They do have a strong tendency to form hydrogen bonds, possibly with polar regions of surfactants. Lecithin has a long history of use in the confectionery industry to control viscosity and reduce stickiness (see Chapter 9). Inverse gas chromatography has shown that lecithin and polyglycerol polyricinoleate (PGPR) modified the surface of sucrose particles to make it more lipophilic (Rouset et al. 2002). Sugar particles, concentrated in oil dispersions, were found to interact with one another (Bahm et al. 2006); adsorption of lecithin to the surface of dispersed sugar particles modified the attractive forces between the sugar particles (Babin et al. 2005). Water bridging and minor components also influence these forces (Gaonkar 1989; Johansson and Bergenstahl 1992c). Water vapor permeability through confectionery coatings is also strongly affected by composition (Ghosh et al. 2005). Surfactants, such as lecithin, PGPR, and monoolein inhibit

these interactions, resulting in decreased viscosity and sedimentation (Johansson and Bergenstahl 1992a, b; Servais et al. 2004). Sugar particles may also serve as heterogeneous crystallization nuclei for confectionery fats (Aronbine et al. 1988; Dhonsi and Stapley 2006). Recent work has also determined differences in the magnitude of interactive forces in butterfat, cocoa butter, and lauric fats (Dickinson et al. 2005). Saccharides can compete with mesophase-forming surfactants for available water. Functional properties are often modified by this competition.

4.6 Surfactant/Hydrocolloid Interactions

Hydrocolloids also referred to as gums, have been widely used in the food industry as thickeners and agents for gel formation and particle suspension (Belitz et al. 2004b). They work cooperatively with surfactants to stabilize emulsions against flocculation and coalescence. Surfactants adsorb at the interface to provide steric and electrostatic stabilization. Hydrocolloids, by increasing the viscosity of the aqueous phase, retard the mobility of dispersed phase droplets. For convenience, cellulose will be included in this discussion.

Hydrocolloids have very weak or no surface activity. Some of these products have no lipophilic groups in their molecular structure. However, some gums, such as guar and arabic, are surface-active because they contain a few percent of proteins, which contain some lipophilic amino acids. Others, such as pectin, contain small lipophilic groups bound to the polymeric chain by ester and ether linkages. Starches and hydrocolloids are chemically modified to include nonpolar functionality (Table 4.4). Surfactant/hydrocolloid interactions may be explained by competition for the interface (Garti et al. 1999).

Table 4.4 Common chemical modifications to starches and hydrocolloids

Product	Added group	Typical applications
Starches		
Ethers	$-OCH_2CHROH$	Thickeners for refrigerated and canned foods, pie fillings
Carboxymethyl	$-OCH_2CO_2H$	Instant gelling products
Starch Esters	$-OPO_3H-OCO(CH_2)4COO-$	Improved freeze-thaw stability, soups, bakery products, sauces
Cross-linked	Phosphates, dicarboxylic acids	Products requiring stability at extremes of pH
Celluloses		
Alkylated	$-OCH_3'$ $-OCH_2CH_3'$	Viscosity rises with temperature, batter, dehydrated fruits, coatings
Carboxymethyl	$-OCH_2CO_2H$	Jellies, fillings, ice cream, bakery products, dehydrated foods
Hydrocolloids		
Propylene glycol alginate	$-OCH_2CH(CH_3)OH$	Suspending agent, salad dressings

Polar hydrocolloids may interact with the hydrophilic functional group of a surfactant through ionic or hydrogen bonds (Babak et al. 2000). Some of these complexes have been utilized to reduce total fat and to replace saturated fats with liquid oils (Reimer et al. 1993). The existence of these complexes is more difficult to establish than starch inclusion complexes. SEM and TEM showed significant strand thickening for monoacylglycerol/starch gels but not monoacylglycerol/cellulose gels. The blends, however, did provide texture and flavor advantages in fat-free products (Baer et al. 1991). Emulsifier interactions with gums in a food system with ice cream ingredients led to more viscous and more pseudoplastic solutions (Parvar and Razavi 2012). Surfactant/hydrocolloid compositions are optimized in wheat bread formulations (Fast and Lechert 1990; Mettler 1992).

4.7 Summary

Amphiphilic molecules are capable of interacting with simple saccharides, starches, and carbohydrates. Ionic, hydrogen, and/or hydrophobic bonding may form carbohydrate/surfactant complexes. A special example of hydrophobic (lipophilic) bonding is the formation of starch/surfactant inclusion complexes. These interactions may have a significant impact on the functional properties (such as stickiness, viscosity, crystallization, or gel strength) of carbohydrates. The fact that these interactions have been and continue to be studied with increasingly sophisticated methods highlights that there is still more to be learned about the interactions between emulsifiers and starch. Modern methods of colloid and emulsion science have led to description of bimolecular interactions. However, correlation of these data to ingredient behavior in complicated food formulations can be quite difficult. This is particularly true when other surface-active agents or polyvalent ions are present. There is, especially, an on-going need to refine predictive cause-effect relationships for practical application parameters in complex food matrices. Application of experimental design will be a useful tool to explain these effects in real foods.

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Chapter 5
Protein/Emulsifier Interactions

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5.1 Introduction

Many food emulsions are more complex than a simple colloidal dispersion of liquid droplets in another liquid phase. The dispersed phase can be partially solidified or contain a lyotropic liquid crystalline phase. The continuous phase may also contain crystalline material, as in ice cream. However, all emulsions are (thermodynamically) unstable. The four main mechanisms that can be identified in the process of destabilizing an emulsion are creaming, flocculation, coalescence, and Ostwald ripening. There are two ways in which the destabilizing process of an emulsion can be influenced. First, use of mechanical devices to control the size of the dispersion droplets and second, the addition of stabilizing additives like low molecular weight emulsifiers or polymers to keep it dispersed. The main purpose of the stabilizer is to prevent the emulsion droplets flocculating and from fusing together (coalescence). Since inter-particle interactions are determined mainly by the droplet surface, the coating with emulsifiers, often surface-active components of biological origin like proteins, mono- and diglycerides, fatty acids, or phospholipids, will be chosen so that the repulsive droplet/droplet interactions increase. The forces most commonly

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observed are electrostatic double layer, van der Waals, hydration, hydrophobic, and steric forces. They are responsible for many emulsion properties including their stability.

The complex mechanisms involved in formation, stabilization, and destabilization of emulsions make fundamental studies on applied systems challenging. One approach has therefore been to clarify the basic physical and chemical properties of emulsions by the study of simpler model systems. The adsorption behavior of single-emulsion components like proteins, fatty acids, surfactants, or phospholipids at liquid/air or liquid/liquid interfaces give information about surface activity, adsorbed amounts, kinetics, conformation, and surface rheology. The development of experimental techniques has made it possible to extend these studies to multicomponent systems. This has provided further information concerning competitive adsorption, displacement, and complex formation, which can be related to emulsion and foam stability.

For further information concerning the physicochemical factors affecting the emulsion structure as well as characterization of food emulsion stability, the reader is referred to the reviews of (Bos and van Vliet 2001; Dickinson 1996, 2003; Dickinson and Stainsby 1982; Wilde 2000; Benichou et al. 2002), and for the principles of emulsion formation to the book of Walstra (2002) along with the other chapters in this book. In this chapter we will focus on the molecular interactions between proteins and other surface-active components present at the interface of the emulsion droplets. Understanding the interaction between these emulsifier components is key to emulsion stability, and opens the possibility to tailor the structure of these systems. Various surface-active components like lipids, low molecular weight (LMW) surfactants, and even phospholipids are here regarded as emulsifiers. We will first discuss the stability of the protein in solution, which is an important factor for their behavior in emulsion systems. Although the behavior at liquid/liquid and liquid/air interfaces can be best compared with the situation in an emulsion or foam, we will also discuss some relevant studies concerning the solid/liquid interface as well as the effect of emulsifiers on the solution behavior of proteins. For completeness we should mention that recent years have seen an increase use of edible particle, like oleosins, protein gel particles, fat crystals and starch granules, as means to stabilize emulsions and foams by the Pickering mechanism (Rayner 2015). This is an expanding field of research, even from the fundamental point of view, beyond the scope of this chapter.

Surface tension measurements are traditionally used to study protein-lipid interaction, cf. (Nishikido et al. 1982; Ericsson and Hegg 1985; Fainerman et al. 1998; Miller et al. 2000a; Vollhardt and Fainerman 2000; Gallier et al. 2012). However, it must be born in mind that any impurity with higher surface activity than the studied components will accumulate at the interface giving a lowering of the surface tension (Miller and Lunkenheimer 1986; Lunkenheimer and Miller 1987; Lunkenheimer and Czichocki 1993) and thus affect data interpretation. As an example, the presence of impurities, e.g. fatty acids, bound to β-lactoglobulin did have a profound effect on the interfacial behaviour of mixtures with Tween 20, as judged from surface elasticity measurements at the air-aqueous interface (Clark et al. 1995). It was

observed that the film containing purified β-lactoglobulin could maintain a more rigid film, at a much higher concentration of Tween 20 as compared to the sample containing impurities. A number of other techniques can also be used to study protein-emulsifier interactions, including surface film balance, ellipsometry, Brewster angle microscopy (BAM), circular dichroism (CD), differential scanning calorimetry (DSC), surface rheology, fluorescence spectroscopy, and neutron reflectivity. It is beyond the scope of this chapter to discuss these techniques in detail, but when necessary a brief explanation will be given.

The link between the molecular interactions between emulsifier components and the properties of food emulsions will be discussed in the last section of this chapter.

5.2 Properties of Proteins and emulsifiers

5.2.1 Protein Structure and Stability

Relevant aspects of protein aggregation and unfolding are briefly discussed as well as the effects of protein structure (random coil proteins versus globular).

An important consequence of protein-lipid interaction is the effect on stability of the protein in solution as well as on its behavior at interfaces. When discussing protein stability, we may distinguish between the conformational stability of proteins and aggregation/precipitation phenomena due to reduced solubility at pH close to the isoelectric point, at high ionic strength (salting out), and/or caused by specific binding of ions (e.g. the formation of calcium bridges) or lipids. Although the two phenomena usually are connected, aggregation/precipitation can occur without major conformational changes of the protein (Tanford 1967). The conformational change of a protein, which has no meaning for proteins lacking secondary structure, can be estimated by circular dichroism (CD) (cf. Creighton 1993), FTIR, compressibility measurement (cf. Gekko and Hasegawa 1986) and calorimetry (cf. Privalov 1979, 1982; Privalov and Gill 1988). The stabilisation of the protein structure have been extensively reviewed by a number of authors (cf. Dill 1990; Creighton 1990; Privalov 1979, 1982; Privalov and Gill 1988; Ponnuswamy 1993), and we will only focus on some aspects of significance in emulsion systems.

The native protein structure is a consequence of a delicate balance of forces including electrostatic forces, hydrogen bonding, van der Waals forces, conformational entropy and so-called hydrophobic interactions (cf. Dill 1990; Privalov and Gill 1988; Ponnuswamy 1993; Pace et al. 1981; Richards 1977). The amino acid sequence of the polypeptide chain (the primary structure) will determine the folding into structural units (the secondary structure) and the association of structural units into domains, tertiary and quaternary structures, gives each protein the unique conformation that is required for its function and activity. Naturally, cross-links, such as disulphide bridges, increase the stability of a protein.

The interior of a globular protein is very densely packed, having a quite constant mean packing density (0.74), a value also found for crystals of small organic molecules (Richards 1977). Thus, van der Waals forces and hydrogen bonding, which are short range interactions, play an important role for the stability of folded proteins (Privalov and Gill 1988). As first pointed out by Kauzmann (1959), it is clear that the so-called hydrophobic interactions play an important role in stabilizing the protein structure. The nonpolar amino acid residues will provide a strong driving force for folding, leading to an accumulation of hydrophobic residues in the core of the protein molecule. The polar amino acid residues (uncharged and charged) will interact favorably with an aqueous solvent and will consequently be located on the outside of the protein. The nature of hydrophobic interactions in this context is not yet fully understood (cf. Dill 1990; Privalov and Gill 1988; Ponnuswamy 1993), since it still is difficult to analyze them separately from other forces contributing to the stabilization of the protein structure (Privalov and Gill 1988).

It is important to bear in mind that proteins are only marginally stable at room temperature. This means that the exchange of only one amino acid residue, by for instance genetic engineering, might affect the protein stability considerably (Matsumura et al. 1988; McGuire et al. 1995b). Lipid, surfactant and denaturing agents such as urea can also affect protein stability. In addition, some proteins have as part of their biological role, specific binding sites for lipids. These binding sites can even be specific for a certain class of lipids. Thus it is important to consider protein-lipid interactions in relation to the features of each individual protein. As discussed extensively by Norde et al. (1986, 2000), Haynes and Norde (1994), the delicate balance between forces that control protein stability might change in the proximity of an interface, leading to unfolding upon adsorption. The lipid-aqueous interface of self-assembled structures is also an important type of interface where unfolding of the interacting protein can occur. The loss of entropy upon protein folding is the main force counteracting the stabilization of the protein structure (Dill 1990). Thus, unfolding upon adsorption is an entropically favoured process (Norde 1986, 2000; Haynes and Norde 1994). Furthermore, at an interface the unfolded hydrophobic domains might be oriented in such a way that their exposure to the aqueous environment is minimized. In fact, Norde argues that the entropy gained by the unfolding of the protein upon adsorption can be a significant driving force for adsorption (Norde 1986, 2000; Haynes and Norde 1994). However, protein adsorption on apolar surfaces could lead to increased secondary structure in a protein as observed for enzymes like α-chymotrypsin and serine proteinase savinase on Teflon (Maste et al. 1997; Zoungrana et al. 1997; Norde 2000). A further consequence is that the presence of lipid bilayers, and as well as hydrophobic surfaces in general can accelerate peptide aggregation, although the mechanism is not yet understood fully understood (Campioni et al. 2014; Vacha et al. 2014).

The folding and unfolding of proteins have been shown, under certain conditions, to occur via an intermediate state, the so-called molten globule state (Dolgikh et al. 1981, 1985; Ohgushi and Wada 1983; Kuwajima 1989; Ptitsyn et al. 1990; Dickinson and Matsumura 1994). This state, which is somewhere between the native and completely unfolded state, is characterized by a retained secondary struc-

ture, but with a fluctuating tertiary structure. The protein molecule is also more expanded and is exposing more hydrophobic domains. The molten globule state is hard to detect by calorimetric measurements, since the unfolding of the molten globule is accompanied with little or no heat absorption (Kuwajima 1989). As discussed by Dickinson and Matsumura (Dickinson and Matsumura 1994), the molten globule state can be achieved in a number of ways, as pH-changes, increase of temperature, the use of denaturation agents, breaking of disulphide bridges and removal of ligands or co-factors bound to the protein. For instance it has been reported that the calcium free form of α-lactalbumin is more hydrophobic (Lindahl and Vogel 1984). It is noteworthy that not all protein native states are the most thermodynamically stable state for a protein. Indeed, the native state can in some cases be considered a metastable state and a protein can transition into more stable non-native states with varied tertiary and secondary structure upon overcoming an energy barrier, which are pre-requisites for amyloid formation (for a recent review see (Knowles et al. 2014)).The formation of amyloid aggregates have been demonstrated for a range of milkproteins, like κ-casein (Ossowski et al. 2012; Thorn et al. 2005; Léonil et al. 2008) and β-lactoglobulin (Dunstan et al. 2009). Formation of these types of aggregates has technological implications for food processing when this involves e.g. elevated temperature and shear forces. One can here imagine both negative and positive effects on the structure and texture of e.g. dairy products like yogurt and cheese.

Proteins might also adopt a molten globule state when interacting with an interface. In fact, it was found that α-lactalbumin was more surface active under conditions where it exists in the molten globule state (Engel et al. 2002). This is demonstrated in Fig. 5.1 showing that the adsorption of α-lactalbumin is enhanced as pH is reduced so that the protein structure tends towards that of the molten globule state. It has been proposed that the molten globule state of the protein may be required for the translocation of proteins across biological membranes (Bychokova et al. 1988; van der Goot et al. 1991). The importance of the protein structure in this context was provided by Hanssens and Van Cauwelaert (1978), who studied the penetration of α-lactalbumin in monolayers of DPPC and cardiolipin at physiological pH (pH 7.4) and at pH 4.6 with and without calcium. Indeed, penetration occurred at low pH, when the protein is supposed to be in the molten globule state and was prevented if the protein was adsorbed from a calcium solution (Hanssens and Van Cauwelaert 1978). The conformation of the protein does not always change significantly when interacting with the lipid monolayer. By recording CD-spectra for β-lactoglobulin, α-lactalbumin or BSA bound to mixed monolayers of POPC and POPG and transferred to a quartz plate, Cornell et al. showed (Cornell and Patterson 1989; Cornell et al. 1990) that the protein bound to the lipid monolayer was similar to the one recorded in solution. However, the interaction between lipids and proteins might induce major changes in the lipid packing (see Sect. 5.3.3) and the lipid membrane permeability (Ruggeri et al. 2013).

Protein properties as conformation, charge distribution, association and activity are also strongly influenced by environmental condition, e.g. pH, ionic strength, type of ion and temperature. In this context it is important to point out the effect

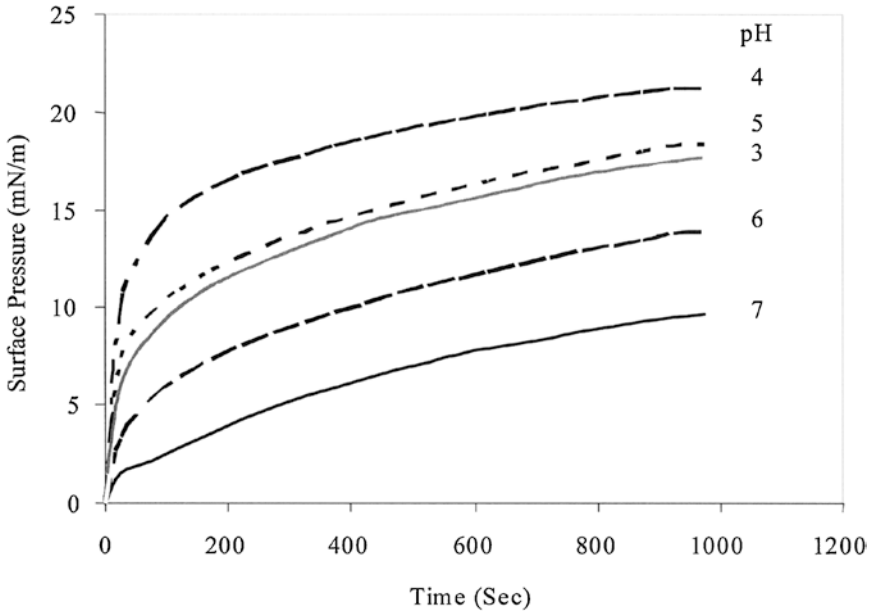

Fig. 5.1 Surface pressure of adsorbing α-lactalbumin as a function of solution pH. The increase in adsorption rates as pH is reduced is initially due to reduction in inter-molecular repulsion as the pH approaches the iso-electric point for α-lactalbumin (pH 4.2). Below this pH, the enhanced adsorption is increasingly due to molecular unfolding as the protein structure tends towards the molten globule state

of type, valence and ionic strength of added electrolyte. As discussed by Ninham et al. this can have profound effect on interactions involving proteins and other polyelectrolytes, in particular under physiologically relevant conditions (Boström et al. 2001, 2002; Ninham 2002). They argue that the present theory is not adequate to distinguish between van der Waals interactions and electrostatic interactions under these conditions. When trying to understand the driving forces for protein adsorption at different surfaces, it is important to remember that the surface of the protein itself is not homogeneously charged. On the contrary, "charged patches" occur that give effective surface areas carrying opposite charges within the protein surface, and this explains why the driving force for protein adsorption at surfaces of the same charge is stronger than to uncharged surfaces (Jachimska and Pajor 2012; Ruggeri et al. 2013) The same concept explains interactions between proteins and electrolytes bearing the same overall charge (Kayitmazer et al. 2013).

5.2.2 *Emulsifiers and Their Phase Behavior*

Different types of emulsifiers are defined (1) aqueous soluble, surfactant type and (2) lipids with low aqueous solubility. The self-assembled structures formed by the different types of surfactants are discussed.

Lipids can be divided into two major groups: polar and nonpolar lipids. The nonpolar lipids, primarily the triglycerides, have small polar groups, and hence show only limited interaction with aqueous systems. However, recent study has shown that there might be some uptake of water in triglycerides like triolein, forming clusters of water in the oil phase (Stamm et al. 2018). The polar lipids, however, with large charged or uncharged polar groups, giving these lipids amphiphilic nature, associate in aqueous systems. The common feature for the self-assembly of the polar lipids in aqueous environment is the formation of a polar interface that separates the hydrocarbon and water regions. The hydrocarbon chains can exist either in a fluid state, as in liquid crystalline phases, or in a solid state, as in the lipid gel phases (Larsson 1994). Generally, the melting of the chains in an aqueous environment occurs at a much lower temperature compared to the melting of the pure lipid.

It is convenient to distinguish between surfactants/polar lipids according to their water solubility:

1. Polar lipids and synthetic analogues, i.e. surfactants, that are water soluble in monomeric and micellar form,
2. Polar lipids with very low water solubility, but with the ability to swell into liquid crystalline phases.

The water-soluble polar lipids (e. g., ionised fatty acids, bile salts, and synthetic surfactants, charged or uncharged) have monomeric solubility in the millimolar range or for some non-ionic surfactant even in the micromolar range and forms micelles at higher concentrations. The critical micelle concentration (cmc) is considered to be a narrow concentration range, within which aggregates start to form by a strong co-operative process (Lindman and Wennerström 1980). The driving force for micelle formation is the hydrophobic interaction (cf. Tanford 1980). The cmc for single-chain amphiphiles decreases with increasing chain length; and for ionic amphiphiles cmc also depends on the ionic strength, as addition of salt reduces the electrostatic repulsion between the charged head groups. Increased temperature has, however, only a moderate influence on cmc, once the temperature has exceeded the critical temperature, where the monomer solubility is equal to the cmc (Krafft temperature).

A common feature of the two classes of polar lipids is the tendency to form lyotropic liquid crystalline phases. A summary of some of the different liquid crystalline phases that can occur is given in Fig. 5.2. With decreasing water content, the phase behavior of polar lipids often follows the sequence: hexagonal phase (H_I) → lamellar phase (L_α) for water soluble lipids and lamellar phase (L_α) → reversed hexagonal phase (H_{II}) for lipids with low water solubility. At low water content an

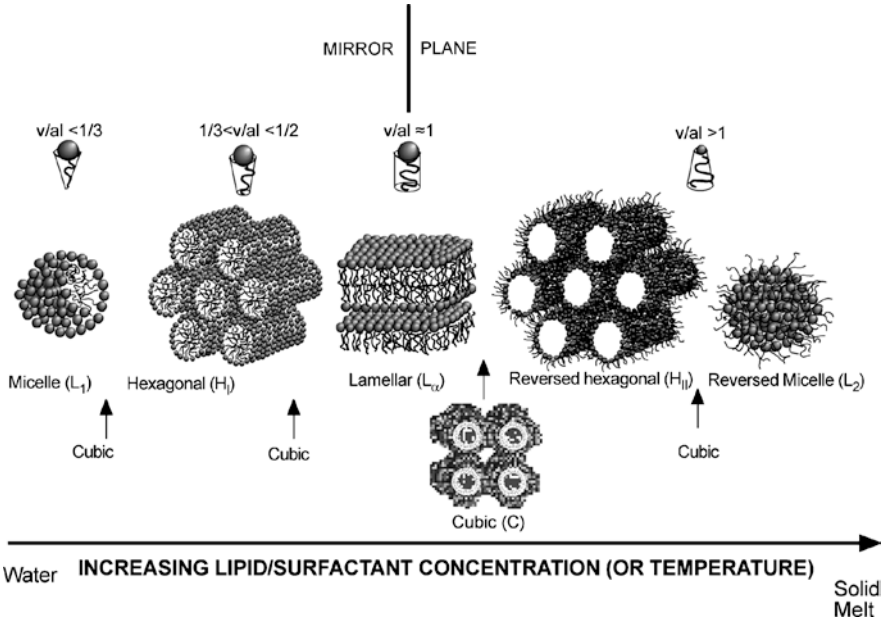

Fig. 5.2 Commonly formed association structures by polar lipids. Phase transitions can be induced by changes in water content, temperature or by interaction with other solution components, like proteins. The lamellar liquid crystalline phase (L_α) can be regarded as the mirror plane, where the aggregates are of the "oil-in-water" type on the water rich side and of "water-in-oil" type on the water poor side (Fontell 1992). On both the water rich and water poor sides of the L_α there are two possible locations for cubic phases. Other "intermediate phases" may also occur. The formation of a particular phase can in many cases be understood by looking at the geometric packing properties of the amphiphilic molecule in the particular environment (Israelachvili et al. 1976; Mitchell and Ninham 1981). This property can be expressed by the so called packing parameter (*v/al*), which is defined as the ratio between the volume of the hydrophobic chain (*v*) and the product of the head group area (*a*) and the chain length (*l*)

inverse micellar structure, the L_2 phase, is formed, in which the hydrocarbon chains form the continuous medium and the aqueous medium is present within the micelles. Cubic liquid crystalline phases (Q) often occur in between these phases. Phase transitions can also occur with changes in temperature; with increasing temperature the sequence of thermal transitions is usually the same as with decreased water content. The formation of a particular phase can in many cases be understood by looking at the geometric packing properties of the amphiphilic molecule in the particular environment (Israelachvili et al. 1976; Mitchell and Ninham 1981), that is the cross-section area of the polar head group in relation to that of the acyl chain. This property can be expressed by the so called packing parameter (*v/al*), which is defined as the ratio between the volume of the hydrophobic chain (*v*) and the product of the head group area (*a*) and the chain length (*l*). The packing parameter for a particular environment will determine the curvature of the interface and thus the particular phase. Generally speaking (see Fig. 5.2), a value of the packing parameter

lower than unity (cone shaped amphiphile) facilitates the formation of structures where the polar interface is curved towards the hydrocarbon phase, i. e. structures of "oil-in-water" type (L_1, H_I). On the other hand a value larger than one (reversed cone shaped amphiphile) will give the reverse curvature and favor "water-in-oil" structures like H_{II} and L_2. When the packing parameter is changed by for instance changes in ionic strength, temperature or addition of other molecules like proteins, phase transitions will ultimately arise. Increased temperature, e. g., will increase chain mobility and thereby increase the volume of the lipophilic part of the molecules, explaining the often seen thermally induced transition $L_\alpha \rightarrow H_{II}$. Decreased hydration will decrease the head group repulsion, resulting in a decreased interface area and thus in an increase of the packing parameter.

In nature and in many technical applications, the lipid aggregates consist of a mixture of different lipids that either exist in a homogenous mixture or separate into domains. As discussed in the review by Raudino (1995), the lateral distribution in these mixed aggregates is influenced by a number of factors like ionic strength, presence of polymers/proteins as well as the composition of the lipids and it is thus hard to give any general rules to predict when phase separation will occur.

Luzzati and co-workers determined the main features of the most commonly found mesophases in the early 1960s by X-ray diffraction (reviewed by Luzzati (1968)). Results from spectroscopy studies have increased the understanding of the dynamic nature of these phases. The lamellar phase (L_α) consists of stacked infinite lipid bilayers separated by water layers, while the hexagonal phases consists of infinite cylinders, having either a hydrocarbon core (H_I) or a water core (H_{II}). As shown in Fig. 5.2, the cubic phases (C) can exist in several locations in the phase diagram and have been shown to exist in a number of lipid systems (Fontell 1990; Templer 1998). They are isotropic and highly visco-elastic. Different structures of the cubic phases, depending on the particular lipid system, have been suggested (Luzzati et al. 1968; Larsson 1989; Lindblom and Rilfors 1989; Fontell 1992; Hyde et al. 1997; Templer 1998):

1. *The bicontinuous cubic phase* that consists of curved non-intersecting lipid bilayers, forming two unconnected continuous systems of water channels, was first discovered by Larsson and co-workers (cf. Lindblom et al. 1979; Larsson 1989; Templer 1998). If an interface is placed in the gap between the methyl end groups of the lipid in the bicontinuous bilayer type of cubic phase, it will form a plane that can be described as a minimal surface (Larsson 1989; Andersson et al. 1988). This type of cubic phase, C_{bic}, has been observed in aqueous dispersions of polar lipids with low aqueous solubility like monoglycerides, phospholipids and glyceroglucolipids (Larsson 1989, 1994; Fontell 1990), phytantriol (Barauskas and Landh Langmuir 2003, 19, 9562–9565) as well as for water soluble surfactants like ethoxylated fatty alcohols (Wallin et al. 1993).
2. *The discrete type of cubic phase* was first suggested by Luzzati et al. (1968). The occurance of micellar cubic phase, C_{mic}, where disjointed reversed micelles embedded in a three-dimensional hydrocarbon matrix are organized in a cubic symmetry, space group Fd3m, has been reported by Luzzati and co-workers

(1992). The formation of this type of C_{mic} phases was reported for aqueous systems containing monoolein and oleic acid (Mariani et al. 1988, 1990; Luzzati et al. 1992; Borné et al. 2001), for aqueous mixtures of sodium oleate and oleic acid (Seddon et al. 1990), and consequently also during lipase catalysed lipolysis of monoolein in aqueous dispersions under neutral/alkaline conditions (Caboi et al. 2002; Borné et al. 2002a).

Today, cubic lipid-aqueous phases are recognized as important in biological systems (Mariani et al. 1988; Larsson 1989, 1994, 2000, 2002; Lindblom and Rilfors 1989; Seddon 1990; Landh 1995; Luzzati 1997; Hyde et al. 1997; De Kruijff 1997; Templer 1998; Pomorski et al. 2014; Almsherqi et al. 2009; Deng et al. 2009). Some of these reports suggest that cubic lipid-aqueous phases can occur during the fusion of biological membranes (Chernomordik 1996). There are a vast amount of studies of membrane fusion (c.f, the comprehensive reviews by (Kinnunen and Halopainen 2000; Epand et al. 2015; Goñi 2014), which is impossible to cover here. The liquid-crystalline lipid aqueous phases can exist in excess of aqueous solution. One example of such lipid dispersions is the thermodynamically unstable vesicles or uni- or multilamellar vesicles,[1] which are formed from lamellar (L_α), phases. The stability, size and shape of vesicles can vary, depending on the composition of lipids and aqueous phase (for reviews see for instance (Helfrich 1989; Lasic 1993; Komura 1996; Lasic et al. 2001)). In analogy with liposomes, dispersions of cubic lipid-aqueous phases, cubosomes, first discovered by Larsson et al. (Larsson 1989, 2000), Landh (1994) can form in excess of water. The stability of cubosomes, formed in monoolein -H_2O-based systems, and the corresponding dispersed H_{II} phase (hexosomes) in the monoolein-triolein-H_2O system were found to increase in the presence of an amphiphilic block-copolymer (polyoxamer) (Landh 1994; Gustafsson et al. 1996, 1997). Since the early work of Larsson et al. several studies on different types of dispersed liquid-crystalline nanoparticles have been presented with focus on systems for drug delivery as well as delivery of functionality to foods (Vandoolaeghe et al. 2006; Barauskas et al. 2005a, b, 2006a; b; Sagalowicz et al. 2006a, b; Johnsson et al. 2006; Tamayo-Esquivel et al. 2006; Boyd et al. 2006; Almgren and Rangelov 2006; Angelov et al. 2006; Yaghmur et al. 2006; Worle et al. 2006; Spicer 2005a, b; Esposito et al. 2005).

5.3 Protein/Emulsifier Interaction in Solution

5.3.1 Aqueous Soluble –Surfactant Type of Emulsifiers

The monomer concentration (defined by cmc) is an important parameter for the interaction between the emulsifier and the protein.

[1] The term liposomes is according to IUPAC recommendation synonymous to lipid vesicles, but is sometimes used for multilamellar vesicles.

Ionic surfactants interact with most proteins, even for surfactant and protein systems bearing the same net charge (Santos et al. 2003). High surfactant concentrations will generally lead to unfolding of the protein structure. The interactions between non-ionic surfactants and proteins are weaker and seldom affect the structure of proteins. Several reviews concerning the interaction between water-soluble polar lipids and protein are focused on the interaction between ionic surfactants, e. g. sodium dodecylsulphate (SDS), and globular proteins at low and intermediate temperatures (Steinhardt and Reynolds 1969; Lapanje 1978; Makino 1979; Jones and Brass 1991; Ananthapadmanabhan 1993; Dickinson 1993, 1999; Bos et al. 1997). Protein interactions with lipids have also been explored with the focus to understand not only changes in protein conformation but also the effect on lipid packing as discussed in Sect. 5.3.3 (De Kruijff and Cullis 1980; Minami et al. 1996; Ruggeri et al. 2013; Simidjiev et al. 2000; Zuckermann and Heimburg 2001; Lendermann and Winter 2003; Fuertes et al. 2010). Since vast amount of the surfactant-protein work is devoted to SDS, we will use this system as an example and at the end of this section we will discuss some exceptions.

We can distinguish between two types of binding of surfactants to proteins:

1. A high affinity type of binding that occurs at low lipid concentration (Jones and Brass 1991)
2. Non-specific co-operative interaction taking place at higher concentrations (Jones and Brass 1991; Ananthapadmanabhan 1993).

An example of a binding isotherm, where the two types of binding occur, is given in Fig. 5.3. In this isotherm, for the binding of sodium dodecylsulphate (SDS) to the net positively charged lysozyme, the regions of high affinity non-co-operative binding, at low surfactant concentration, is well separated from the co-operative binding observed at higher concentration. Similar binding isotherms are obtained for SDS and the net negatively charged albumin (Santos et al. 2003). For comparison an example of a binding isotherm for the binding of a nonionic surfactant, n-octyl-β-glucoside, to the same protein, is also inserted. In this case only non-specific co-operative binding occurs.

5.3.1.1 Specific Binding of Proteins and Emulsifiers

The specific binding is mediated by ionic and hydrophobic interactions and usually occurs below the cmc of the surfactant (Jones and Brass 1991; Jones and Manley 1979, 1980; Yonath et al. 1977a, b). There are many examples of proteins that possess binding activity, including bovine serum albumin and β-lactoglobulin. Investigation of the binding properties of these proteins has been generally confined to studies in bulk solution. For example, the presence of a fluorescent tryptophan residue in the hydrophobic cleft of β-lactoglobulin (Papiz et al. 1986) has facilitated the study of emulsifier binding by fluorescence titration. Subsequent analysis of binding by conventional methods such as that of Scatchard (1949) allows determination of the dissociation constant (K_d) of the complex formed. Typical

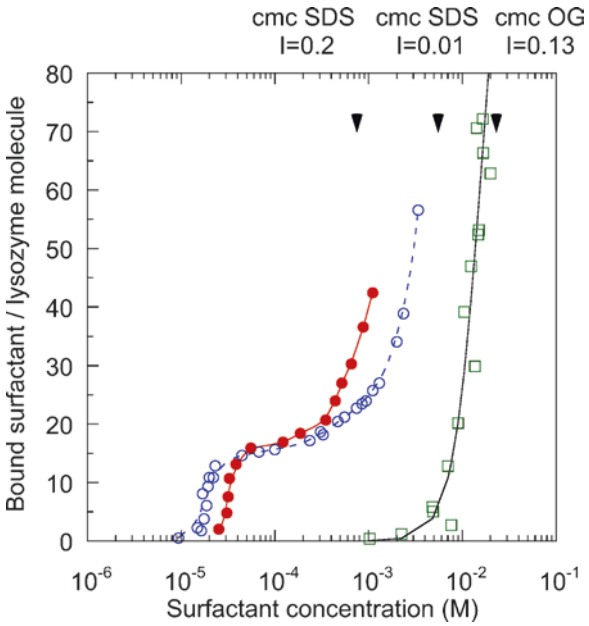

Fig. 5.3 Binding isotherms for binding of surfactants to lysozyme in aqueous solution at 25 °C. The isotherms (○-Blue, ●-Red) for sodium dodecylsulphate (SDS) have regions of both high affinity non-co-operative binding, at low surfactant concentration, and co-operative binding at high concentration. The influence of ionic strength on the binding isotherm is shown: ●-Red, ionic strength (I) 0.0119 M and ○-Blue, ionic strength 0.2119 M at pH 3.2. For comparison, an example of a binding isotherm where only nonspecific co-operative binding occurs, is also inserted. This isotherm, describing the binding of the nonionic n-octyl-β-glucoside (OG) to lysozyme (□-Green) was measured at pH 6.4, ionic strength 0.132 M. The protein concentration was 0.13% w/v. The arrows indicate cmc for the different surfactants and ionic strengths. The data is adopted from Jones (Jones and Brass 1991) and the experimental details are given in references (Jones et al. 1984) and (Jones and Brass 1991) for SDS and OG, respectively

examples of K_d's for β-lactoglobulin are shown in Table 5.1. The effect of complex formation can usually be detected by shifts in the surface-tension (γ) curve (Dickinsson and Woskett 1989). An example of this is shown for Tween 20 and β-lactoglobulin in Fig. 5.4 (Coke et al. 1990). Surface-tension/concentration (γ-c) curves for Tween 20 alone and in the presence of a fixed concentration of β-lactoglobulin (0.2 mg/ml; 10.9 μM) are shown.

The general features described earlier are evident with a comparatively low concentration of protein causing a significant reduction in γ. In the absence of protein, γ reduces gradually with increasing Tween 20 concentration. The gradient of the reduction in surface tension reduces at higher Tween 20 concentrations (>30 μM) but doesn't become completely flat due to failure to attain equilibrium γ, possibly due to the presence of a mixture of surface-active species in the Tween-20 sample. In contrast, the curve in the presence of protein maintains a relatively steady surface-tension value of about 50 mN/m up to Tween-20 concentrations of 25 μM due

Table 5.1 Typical dissociation constants of emulsifier/β-lactoglobulin complexes

Emulsifier	Dissociation Constant	Reference
Tween 20	4.6 μM	Clark et al. (1993)
L-α lysophosphatidyl-choline, palmitoyl	166 μM	Sarker et al. (1995)
Sucrose monolaurate	11.6 μM	Clark et al. (1992)
Sucrose monostearate	1.02 μM	Clark et al. (1992)
Sucrose monooleate	24.8 μM	Clark et al. (1992)
Sodium stearoyl lactylate, pH 7.0	0.26 μM	Clark, unpublished
Sodium stearoyl lactylate, pH 5.0	0.30 μM	Clark, unpublished
Lauric acid	0.7 μM	Frapin et al. (1993)
Palmitic acid	0.1 μM	Frapin et al. (1993)

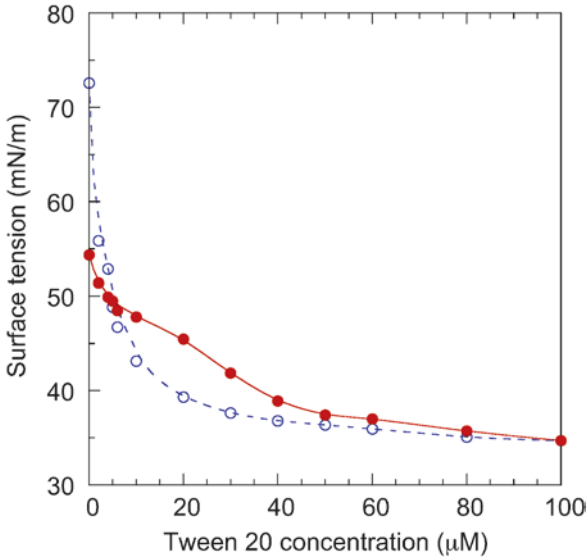

Fig. 5.4 Surface tension isotherm for Tween 20 in the absence (○-Blue) and presence (●-Red) of 0.2 mg/mL β-1actoglobulin. The data were recorded after 20 minutes adsorption and are therefore not at equilibrium

to the surface tension reduction caused by adsorption of the protein. This means that the curve for the sample containing protein crosses that of Tween 20 alone. This is strong evidence for complex formation between the two components, since the curves cross due to a reduction in the concentration of free emulsifier in solution as a fraction of the emulsifier interacts with the protein to form the complex. However, later work offered an alternative solution, in that the surface elasticity of the protein adsorbed layer resisted the adsorption of the surfactant at low concentrations, thus retarding surfactant adsorption, and was dependent on the elasticity of the protein stabilized interface (Mackie et al. 1999) (see Sect. 5.4.2 for more details).

Thus, great care must be taken when considering the surface properties of compounds in solutions containing mixtures of interacting components. In the simplest case of a single binding site, the two-component system becomes a three-component

system comprising free emulsifier, free protein, and emulsifier/protein complex. The relative proportions of the components present can be calculated in the following manner (Clark et al. 1992). In the simplest case, the interaction of an emulsifier (E) with a protein (P) can be described by the expression.

$$\mathrm{P} + \mathrm{E} \leftrightarrow \mathrm{PE} \tag{5.1}$$

where PE is the emulsifier/protein complex. Thus the dissociation constant (K_d) for the complex can be expressed as

$$K_d = \frac{[\mathrm{P}][\mathrm{E}]}{[\mathrm{PE}]} \tag{5.2}$$

where the square brackets indicate molar concentrations of the different species. It is also the case that

$$[\mathrm{P}] = [\mathrm{P_{tot}}] - [\mathrm{PE}] \tag{5.3}$$

$$[\mathrm{E}] = [\mathrm{E_{tot}}] - [\mathrm{PE}] \tag{5.4}$$

where $[\mathrm{P_{tot}}]$ and $[\mathrm{E_{tot}}]$ are the total protein and emulsifier in the system. Substituting Eqs. (5.3) and (5.4) in (5.2) gives

$$[\mathrm{PE}] - \left([\mathrm{E_{tot}}] + [\mathrm{P_{tot}}] + K_d\right)[\mathrm{PE}] + [\mathrm{P_{tot}}][\mathrm{E_{tot}}] = 0 \tag{5.5}$$

which can be solved for [PE] and can be used to calculate the relative concentrations of the three components. In addition, the binding data, which may comprise a change in a parameter (e.g., intrinsic fluorescence) caused by formation of the complex may be fitted using this equation, provided there is a single active binding site and the titration is carried out to saturation. Alternatively, it is possible to determine the dissociation constant and number of binding sites from the Scatchard (1949) equation

$$\frac{v}{[\mathrm{E}]} = \frac{n - v}{K_d} \tag{5.6}$$

where v is the fraction of protein with occupied sites (i.e., $[\mathrm{PE}]/[\mathrm{P_{tot}}]$). If the Scatchard plot of v against $v/[\mathrm{E}]$ gives a straight line, it indicates the presence of only one class of binding sites. The gradient of this line is $1/K_d$, and the intercept on the x axis gives the number of binding sites, n. If the Scatchard plot does not give a straight line, then the shape of the curve obtained can be used to identify if the observed binding is positively or negatively cooperative or the presence of multiple independent sites. In the former case the Hill equation can be used to determine the K_d and a cooperativity coefficient (Hill 1910).

5.3.1.2 Non-specific Interaction

The non-specific interaction often occurs close to the cmc as it is associated with the aggregation of the surfactant and usually leads to a destabilization of the native conformation. The cmc of the surfactant is thus an important parameter and conditions that affect cmc will generally affect the binding, cf. (Ananthapadmanabhan 1993; Waninge et al. 1998). The saturation of all the binding sites generally corresponds to 1–2 g of surfactant per gram of protein (Ananthapadmanabhan 1993; Jones and Brass 1991; Reynolds and Tanford 1970).

The extent of interaction and unfolding depend mainly on the nature of the surfactant hydrophilic group, surfactant chain length, ionic strength, pH, temperature and organic additives as well as on the protein itself (Ananthapadmanabhan 1993). Organic additives include the presence of impurities in proteins as well as in the lipids. For instance, it has been demonstrated by Lunkenheimer and co-workers that commercial SDS samples usually contains a substantial amount of dodecanol, which is more surface active than SDS by itself (Miller and Lunkenheimer 1986; Lunkenheimer and Miller 1987; Lunkenheimer and Czichocki 1993). Similarly, Clark et al. (1995) showed that β-lactoglobulin contains bound fatty acids, which may alter the binding of other surface active compounds. Clearly, the presence of amphiphilic impurities may give anomalous effects on the binding of other surfactants.

The effect of surfactant protein interaction on the structural stability of proteins depends strongly on the mode of interaction. In fact as shown in Fig. 5.5, the same surfactant can act as both stabilizing and destabilizing depending on surfactant concentration as well as other solution conditions. At low surfactant-to-protein ratios, high affinity interaction between certain proteins and surfactants occur. This interaction stabilizes the protein structure against thermally induced unfolding, thus the thermally induced transition is shifted towards higher temperature as illustrated in Fig. 5.5 and previously reported by Hegg (1980) for SDS and β-lactoglobulin. Similar findings has also been reported for other protein-surfactant complexes such as between fatty acids or SDS and bovine serum albumin (Gumpen et al. 1979) as well as between palmitic acid and β-lactoglobulin (Puyol et al. 1994). As discussed above increasing the free surfactant concentration to the cmc give rise to non-specific cooperative binding, which in turn can lead to unfolding of the protein as illustrated in Fig. 5.5 (Waninge et al. 1998). This is in agreement with earlier reports, where total surfactant ratio above 10 moles of SDS per mole of serum albumin or 1 mole of SDS per mole of β-lactoglobulin monomer were observed to cause unfolding of the protein (Hegg 1980; Gumpen et al. 1979).

5.3.1.2.1 Anionic

Surfactants like alkylsulphates or alkylethersulphates interacting with proteins with opposite net-charge, e. g. lysozyme or gelatine, might cause precipitation of the protein-surfactant complex due to neutralisation of the net charge (Jones and Manley

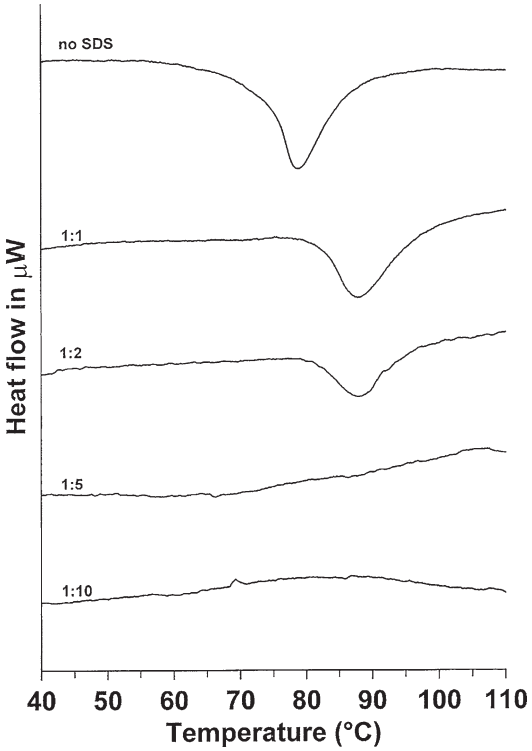

Fig. 5.5 The thermograms from top to bottom shows the thermally induced unfolding of β-lactoglobulin (1.4 mM in 60 mM NaCl, pH 6) when increasing the protein/SDS molar ratio. The cmc of SDS is 0.47 mM at 25 °C and ≈1 mM at 90 °C, when taking into account the ionic strength of the protein solution. Assuming that 1 SDS molecule is bound per β-lactoglobulin monomer, 3 mM SDS has to be added to reach the cmc of the surfactant at 90 °C. The data are adopted from (Waninge et al. 1998), where also the experimental details are given

1979; Fukushima et al. 1981, 1982; Chen and Dickinson 1995a, b; Morén and Khan 1995; Stenstam et al. 2001). Although the protein is precipitated, usually only small changes in the secondary structure occur. At an increased surfactant concentration the complex is dissolved and the protein starts to be unfolded. Generally, denaturation of proteins by long-chain alkyl sulphates such as SDS results in a structure with large fractions of the polypeptide chain in an α-helical conformation (Tanford 1980; Mattice et al. 1976; Jirgensons 1976). As a simple rule, proteins with a low content of α-helix in their native form, such as concanavalin A, β-lactoglobulin and ovalbumin, will increase in α-helix content upon interacting with SDS. The reverse is observed for proteins with a high α-helix content in their native form, e. g. myoglobin and serum albumin (Mattice et al. 1976). The structure resulting from the interaction is thought to consist of helical segments with flexible joints, and with most of the hydrophobic side-chains exposed to the surfactant. The successive binding of SDS opens up the molecules, due to the increased electrostatic repulsion, and unveils new hydrophobic domains, which can bind additional surfactants. This association stabilizes α-helical folding at the expense of non-repetitive structure. The free energy gained by this process in most cases by far exceeds the unfavourable free energy change of disrupting the native conformation (Tanford 1980). Light scattering studies confirm the expansion of the hydrodynamic radius of the protein upon interaction with SDS (Tanner et al. 1982). Several models of the structure of

complexes between SDS and proteins at high surfactant concentration, like the correlated necklace, rod-like structure and flexible helix, have been considered, cf. (Ananthapadmanabhan 1993; Guo and Chen 1990). However, small-angle neutron scattering data strongly indicates a structure resembling a necklace (Guo and Chen 1990; Guo et al. 1990), where the polypeptide chain with high flexibility is decorated with SDS micelles (Mattice et al. 1976; Guo and Chen 1990) as shown in Fig. 5.6. This interaction is reported to take place via the monomeric form of the surfactant (Ananthapadmanabhan 1993; Mattice et al. 1976).

It should also be born in mind that not all proteins are fully unfolded by SDS. For instance it has been shown that the activities of glucose oxidase, papain, pepsin and bacterial catalase were not affected by high concentration of SDS, correlated to the low binding of SDS (Nelson 1971; Jones et al. 1982).

Within the type of surfactant the binding is dependent on the nature of the polar head group, e. g. for anionic surfactant the interaction decreases in the order alkyl sulphates > alkyl sulphonates > alkyl benzene sulphonates > carboxylates ≈ alcohols (Reynolds et al. 1968; Rendall 1976).

5.3.1.2.2 Nonionic

The interaction between nonionic surfactants and proteins are generally weak (Reynolds et al. 1968; Makino et al. 1973; Green 1971; Cordoba et al. 1988; Sukow et al. 1980; Bos et al. 1997). They are therefore often used to solubilise/stabilize proteins in biochemical preparations, e.g. (Ahlers et al. 1990) including membrane

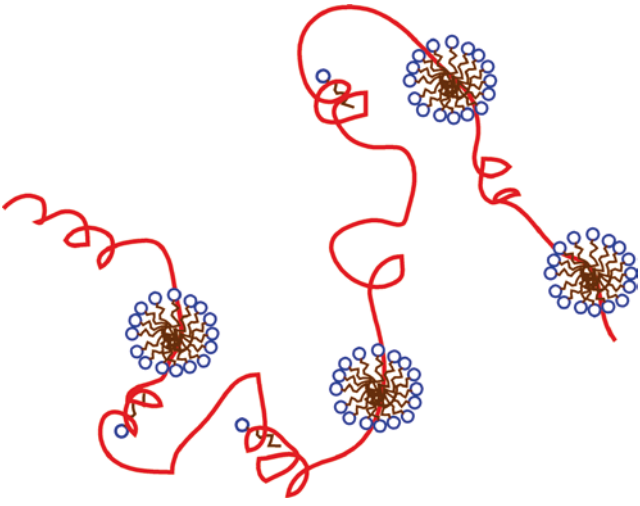

Fig. 5.6 Schematic representation of the so-called necklace model for the interaction between SDS and proteins. The solid line represents the unfolded polypeptide chain, which still contains secondary structure. Micelle-like clusters are co-operatively formed on the polypeptide chain

proteins (Chae et al. 2010). For instance, each β-lactoglobulin monomer binds only one Tween 20 (Wilde and Clark 1993), or one sucrose ester (Clark et al. 1992) or one Triton X-100 (Green 1971). Generally, minor changes of the structure upon interaction are observed (Makino et al. 1973; Cordoba et al. 1988). An unordered, flexible protein, β-casein, was found to bind less than one sucrose ester per protein molecule, possible due to incorporation of the surfactant in β-casein micelles (Clark et al. 1992). The specific ionic interaction present for ionic surfactants in addition to the hydrophobic interaction that leads to more severe effects on the protein structure is absent for the nonionic surfactants (Fig. 5.3). Another reason for the weaker interaction between proteins and nonionic surfactants has been assigned to the lower cmc, which gives fewer monomers in the solution that can bind to the protein (Makino et al. 1973). The cmc is increased when the chain length is decreased, which may change this situation; the binding of octyl glucoside to various proteins was found to occur in a co-operative manner at surfactant/protein molar ratio of hundred and more, without any evidence of protein denaturation (Cordoba et al. 1988).

Also the nature of the nonionic polar head groups will affect the interaction. For a series of Triton X surfactants increasing the hydrophilic oxyethylene chain length was found to decrease the strength of interaction with BSA, due to steric hindrance as well as relatively lower hydrophobicity (Sukow et al. 1980). The calorimetric data indicated that some conformational changes of BSA occurred during the saturation of the low affinity, non-co-operative binding sites (Sukow et al. 1980).

Some studies have also been carried out with the zwitterionic surfactant lysophosphatidylcholine (LPC), which was found to bind co-operatively to puroindoline, a lipid binding protein isolated from wheat flour, at a molar ratio of 5 to 1 (Clark et al. 1993), with an affinity that was dependent on the chain length of the LPC molecule (Husband et al. 1995). One LPC molecule was also found to bind with less affinity to β-lactoglobulin than Tween 20 (Sarker et al. 1995). The binding of Tween 20, as opposed to LPC, had a much more disruptive effect on the interfacial film of the protein, attributed to the bulkier head group of Tween 20. This implies that also a nonionic surfactant can also disrupt the structure of a protein, provided that the binding is strong enough and the hydrophilic head group large enough to sterically induce conformational changes.

5.3.1.2.3 Cationic

Cationic surfactants generally seem to exhibit an intermediate action on water-soluble proteins. Reports in the literature indicate a co-operative interaction with proteins, but with less affinity and thus with less perturbation of the folded state, compared to the effect of the anionic ones (Tanford and Epstein 1954; Kaneshina et al. 1973; Nozaki et al. 1974; Ericsson et al. 1987a, b; Waninge et al. 1998). If the binding is governed both by electrostatic and hydrophobic interactions, anionic and cationic surfactants will obviously occupy different sites. Nozaki et al. has suggested

that the lower affinity of many proteins for cationic compared to anionic surfactants, can be explained by the fact that the cationic arginine and lysine side chains contributes with more CH_2 groups than anionic aspartate and glutamate side chains (Nozaki et al. 1974). This implies that the combined electrostatic and hydrophobic interactions and possibly accessibility of the charged groups will be more favourable for anionic surfactants. As a consequence, the co-operative binding step will start at a higher concentration for cationic relative to anionic surfactants (Ananthapadmanabhan 1993).

5.3.1.3 Effect of Solution Conditions

Increased ionic strength can affect the interaction between protein and ionic surfactants by reducing the electrostatic attraction between surfactants and amino acid residues with opposite net charges. Generally, the high affinity non-co-operative binding is strongly influenced by the electrostatic interaction between surfactant and protein. Thus this part of the binding isotherm will be shifted towards higher surfactant concentration upon addition of salt, as observed for lysozyme and SDS (Fig. 5.3) (Jones and Brass 1991; Jones et al. 1984). Increasing the ionic strength will, on the other hand, favor the co-operative binding by screening the repulsion between the charged surfactant head groups. This part of the surfactant binding isotherm will therefore be shifted towards lower surfactant concentrations, parallel to the decrease of surfactant cmc. Here it is important to point out that the presence of highly charged proteins will affect the formation of micelles as well as the effect of temperature in the same way as a polyelectrolyte. This has been amply demonstrated by Waninge et al. who studied thermally induced unfolding of β-lactoglobulin at a concentration of 1.4 mM in 60 mM NaCl, pH 6, at various molar ratios of SDS and their main findings are illustrated by the thermograms, obtained by differential scanning calorimetry (DSC), in Fig. 5.5 (Waninge et al. 1998). From this figure we note that the peak corresponding to the thermal unfolding disappears when the protein/SDS molar ratio increases above 1:2. This corresponds to a SDS concentration of about 3 mM. The cmc for SDS is about 8.1–8.2 mM in water (Williams et al. 1955; Flockhart 1961). However, the cmc for ionic surfactants decreases with ionic strength and increases with temperature (Williams et al. 1955; Flockhart 1961; Evans et al. 1984b, b) and thus we expect that the presence of β-lactoglobulin (which has a net charge of −5 at pH 7) at a concentration of 1.4 mM in 60 mM NaCl will lower the cmc of SDS to 0.47 mM at 25 °C and ≈1 mM at 90 °C. When taking into account the specific binding of one SDS molecule per β-lactoglobulin monomer, 3 mM SDS has to be added to reach the cmc of the SDS at 90 °C. Thus any affect of non-specific co-operative interaction between the surfactant and the protein is expected to take place at this SDS concentration. In Fig. 5.5 we observe an apparent loss of protein structure. The unfolding of the protein structure at low temperature, which is observed in the presence of most anionic surfactants such as SDS at high concentration, should be maintained at increased temperature. However, since cmc generally increases with temperature,

we might arrive at the situation where the co-operative binding ceases to exist at the high temperature, maybe even below the temperature at which thermally induced unfolding takes place. Interestingly, Waninge et al. (1998) observed that the conformational changes invoked by the non-specific co-operative binding of SDS at 25 °C could be reversed by extensive dialysis.

Although cationic surfactants seem to cause less unfolding of globular proteins at low temperature than anionic, some reports indicate that they can destabilize globular proteins at increased temperature (Ericsson et al. 1983a, 1987a). However, these reports also indicate that the unfolding process at the same time becomes considerably more reversible. The heat denaturation of ovalbumin, which in practice is completely irreversible, was found to be completely reversible in the presence of high concentrations of cationic surfactants (Ericsson et al. 1983a). This was explained by decreased inter- and intramolecular interactions at high temperature, due to interaction between the unfolded protein and surfactant, which facilitates the re-formation of the native complex on cooling. Hansted et al. (2011) investigated in detail the effect of surfactant on the conformation of β-lactoglobulin and the extent of aggregation upon heat treatment by a combination of several techniques including Isothermal titration calorimetry, dynamic light scattering, near-UV CD and tryptophan fluorescence. In their work, they subjected the protein-surfactant solutions to 1 h at 85 °C. They found that all surfactant types investigated interacted at low concentration (1 surfactant/protein) probably due to interactions with the hydrophobic pocket of β-lactoglobulin, and this led to a small change in the tertiary structure of the protein that further stabilize the protein against thermal denaturation. Furthermore, stabilization against heat-induced aggregation also occurred under these conditions for negatively charged surfactants only due to a decrease in the propensity for β-lactoglobulin to form dimers. Cationic surfactants led to further aggregation due to charge neutralization and non-ionic surfactants did not have any effect on the extent of aggregation. At concentrations close to the cmc, partial protein denaturation occurs and the effect of heat treatment on aggregation also depends on the type of surfactant. For anionic surfactants, electrostatic repulsions dominate and little aggregation occurs upon heating. For cationic surfactants, charge neutralization led to further aggregation and for nonionic surfactants no major effects were observed in the extent of aggregation. Finally, well above the cmc, all surfactants lead to stability against aggregation due to the formation of bulk micelles that solubilize unfolded protein segments. Thus it is clear that the mechanism of protein-surfactant interaction depends on the charge of the surfactant and can be driven both by monomeric and aggregate surfactant species.

As a rule of thumb, an increase in pH will shift the binding of anionic surfactants to higher concentrations (Reynolds et al. 1970). In this case one would expect that both the specific and the cooperative binding are affected in the same way. A decrease of pH will have the same effect on binding of cationic surfactants (Subramanian et al. 1984). At low surfactant concentrations, that is, well below cmc, cationic amphiphiles increase the solubility of proteins on the acidic side of the isoelectric point (pI), while precipitation can occur on the alkaline side of

pI. Anionic amphiphiles will affect solubility in the opposite direction. The solubilising effect is also observed at high temperatures.

We conclude that since the binding generally is thought to occur via monomers, any change affecting the cmc will also affect the co-operative binding at concentrations close to and above cmc. Under some conditions the formation of surfactant micelles will be energetically favored compared to binding to the protein. If cmc is of the same order of magnitude as the concentration necessary for binding to occur, the lowering of cmc caused by increasing ionic strength might even prevent binding.

5.3.2 *Phase Behavior of Emulsifier Protein Systems*

So far we have mainly addressed the interaction at low protein concentrations. Morén and Khan (1995) investigated the phase behavior of the anionic SDS, positively charged lysozyme and water over a wide concentration range and one of the phase diagrams they determined is given in Fig. 5.7a. Stenstam et al. (2001) later investigated in detail the stoichiometry of the formed complex and their findings are summarized in Fig. 5.7b. Small amounts of SDS, at a ratio to lysozyme corresponding to charge neutralization of the protein, were found to give precipitation. A net attractive force exists between the surfactant-protein complexes and hydrophobic interactions dominate (Fig. 5.7b). Further addition of SDS dissolved the precipitate and complete dissolution was achieved when the number of SDS molecules was

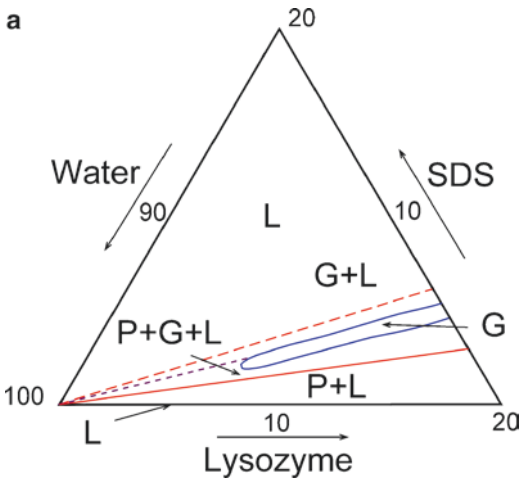

Fig. 5.7 (**a**) Phase diagram of the lysozyme—SDS -water ternary system, where L indicates solution, G gel and P precipitate. The figure is adopted from (Morén and Khan 1995), where experimental details are given. (**b**) Schematic representation of the interaction between protein surfactant complexes in the lysozyme—SDS -water system. Figure adopted from (Stenstam et al. 2001), where the experimental details are given

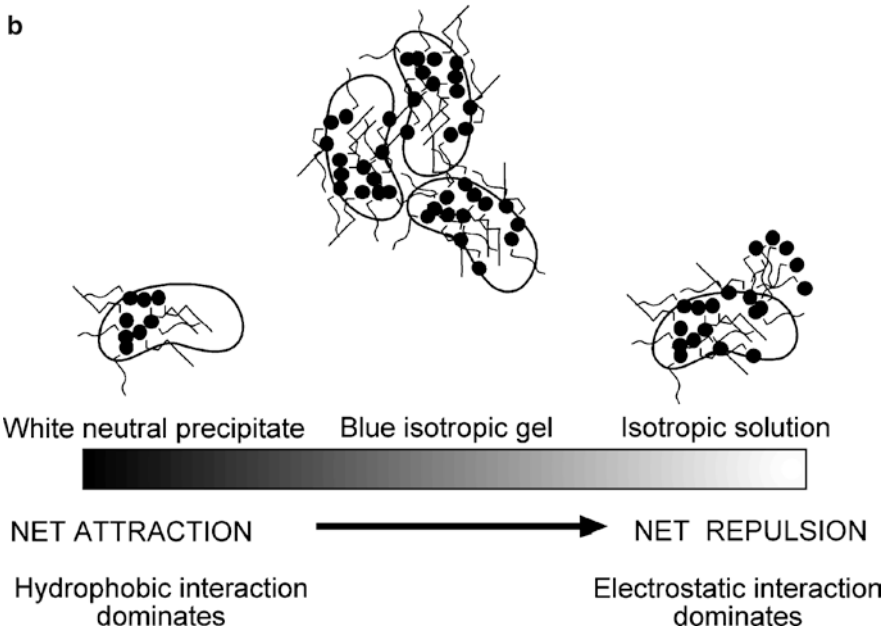

Fig. 5.7 (continued)

equal to the number of (18) positive charges on the protein. A bluish gel phase occurred when the protein concentration was between 7 and 20% (w/w). A further increase of the ratio between SDS and lysozyme, leads to a strong net repulsive electrostatic interaction between the surfactant-protein complexes (Fig. 5.7b). Consequently an isotropic solution is formed. Similar results were found for the oppositely charged system formed by β-lactoglobulin and alkyltrimethyl ammonium chloride surfactants (Hansted et al. 2011). All classes of surfactants were found to modify by β-lactoglobulin heat induced aggregation behaviour. Anionic sodium alkyl sulfate and non-ionic alkyl maltopyranoside were found to reduce aggregation below and around their cmc, respectively. However cationic alkyl trimethyl ammonium chlorides increase the hydrodynamic radius of formed aggregates. The length of the alkyl chain greatly affects the strength of interactions between surfactants and protein. Charged surfactants were found to reduce aggregation in the order C10–C12 > C8 > C14 for sodium alkyl sulfates and C8 > C10 > C12 > C14 > C16 for alkyl maltopyranosides. Alkyl trimethyl ammonium chlorides promote aggregation in the same ranking order as alkyl maltopyranosides reduce it. These differences most likely reflect the fact that anionic and cationic surfactants (as well as the small number of nonionic surfactants that bind below their cmc) bind at different sites on the protein. In this respect cationic surfactants seems to facilitate aggregation both due to structural destabilization as well as neutralizing the protein net-charge. It was also found that the food grade anionic surfactant sodium dodecanoate also inhibited aggregation well below the cmc. Morén and Khan also

investigated the effect of varying alkyl chain length, $C_{12}SO_4$, $C_{10}SO_4$, C_8SO_4, and C_6SO_4 on the lysozyme –sodium alkyl sulfate-water ternary systems (Morén and Khan 1998). The extension of the solution region decreased with increasing surfactant chain length and the surfactant with shortest hydrophobic tail (C_6SO_4) forms the largest solution region with lysozyme without precipitation. The extension of the precipitation region toward higher surfactant concentrations increases with decreasing surfactant chain length. The surfactant concentration required to redissolve the precipitate at dilute protein concentrations therefore seems to follow the cmc for the surfactant in water, which also increases with decreasing surfactant chain length. A single gel phase was only observed for the $C_{12}SO_4$ and $C_{10}SO_4$ systems and not in presence of C_8SO_4 and C_6SO_4. Similar types of gel phases are expected to occur in more food relevant surfactant/lipid and protein aqueous mixtures and therefore offer interesting possibilities to vary the functional properties of foods and food ingredients.

5.3.3 *Emulsifiers with Low Aqueous Solubility*

For emulsifiers with low aqueous solubility the emulsifier self-assembly structure and its properties control the interaction with proteins.

In this section we will discuss interactions involving lipids with low solubility where the lipids exist as dispersed particles, liposomes or vesicles, liquid crystalline phases as well as monolayers at interfaces. Many of the principles discussed in the earlier sections, also do apply for protein-lipid interactions in condensed systems. Polar lipids, which normally are water-insoluble, associate into a variety of structures in aqueous solution. This process will have an impact on interactions with proteins. For lipids with low aqueous solubility the interaction with the proteins mainly involves the self-assembled structure formed by the lipids. However, we note that even polar lipids that are considered water-insoluble have a certain monomer solubility, which although small (about 10^{-7} for monoolein and about 10^{-10}–10^{-12} M for phospholipids) makes it possible for them to interact with proteins in the monomeric form, in particular if the protein has a high affinity binding-site for the lipids. This is demonstrated in Fig. 5.8, which shows the thermograms from differential scanning calorimetry measurements of β-lactoglobulin, distearoylphosphatidic acid (DSPA) and β-lactoglobulin + an aqueous dispersion of DSPA, respectively. The peak corresponding to the thermally induced unfolding transition of β-lactoglobulin in presence of DSPA is shifted towards higher temperature compared to the one recorded for the pure protein. This confirms the presence of a specific interaction between phosphatidic acid and β-lactoglobulin that thermally stabilizes the protein. This was also observed in the presence of dipalmitoylphosphatidic acid (DPPA), but no such interaction was observed when the protein was mixed with phosphatidylcholine, phosphatidylethanolamine or phosphatidylglycerol (Kristensen et al. 1997). The authors also reported the absence of protein-lipid interactions for lipids containing unsaturated fatty acid residues.

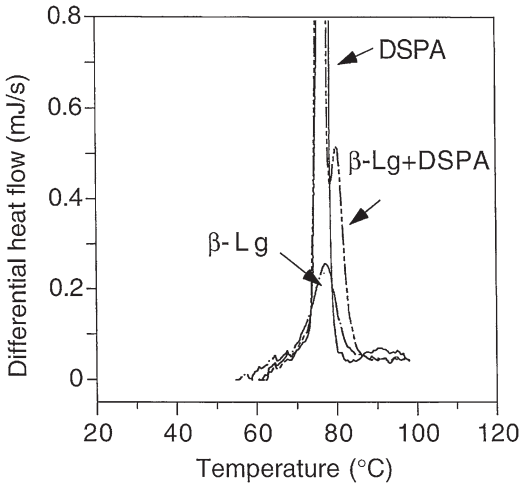

Fig. 5.8 The interaction between distearoylphosphatidic acid (DSPA) and β-lactoglobulin (β-Lg) is demonstrated by the results from differential scanning calorimetry (DSC) where the thermogram of the protein/lipid mixture is compared with those of the pure components. The thermograms of DSPA, 5 % (w/v) (———), β-Lg 5% (w/v) (— · —) and a mixture of β-Lg 5% and DSPA 5% (w/v) (— - - —) in 1% sodium chloride at pH 7. A scanning rate of 10 °C/min was used. Data adopted from Kristensen et al. (1997), where also the experimental details are given

Thus the results show that the interactions between β-lactoglobulin and phospholipids are strongly dependent on the acyl chain as well as the head group. A small negatively charged head group is needed for the interaction to take place. Such an interaction can have important implications for the functional properties of the protein. We discussed above that fatty acids bound to β-lactoglobulin could affect the interfacial behavior of the protein (Clark et al. 1995). Kurihara and Katsuragi (1993) reported that a lipid-protein complex, formed between β-lactoglobulin and phosphatidic acid, could mask bitter taste. This property was suggested to be specific for phosphatidic acid as no effect was observed for mixtures of β-lactoglobulin and phosphatidylcholine, triacylglycerol or diacylglycerol.

Even if no specific interaction occurs, proteins can have an impact on liquid crystalline phase or gel phase due to the limited space of the aqueous cavity. This was demonstrated by Minami et al, who investigated the incorporation of lysozyme, β-lactoglobulin and α-lactalbumin in a sphingomyelin gelphase containing 0.6 wt% sodium palmitate and 80 wt% aqueous solution (Minami et al. 1996). The dimension of the aqueous layer in the gel phase was suggested to limit the amount of protein that could be incorporated. Above this limit, phase separation will occur with a gel phase and an “outside” protein rich solution. The protein will, at high enough concentration, probably also compete for the water in the interlamellar spacing, which eventually leads to a reduction of the aqueous layer thickness. This effect was demonstrated for high molecular weight polymers in equilibrium with the phosphatidylcholine lamellar phase (LeNeveu et al. 1977). The polymer was unable

to enter the aqueous layer, but still exerted an osmotic stress that was large enough to compress the lamellar lattice as shown by x-ray diffraction data. This method has been used to measure the interaction between the lipid bilayers (LeNeveu et al. 1977; Cowley et al. 1978).

When proteins enter into the aqueous layer of a lamellar phase they affect the swelling of the lipid phase. This was shown by Rand (1971), who studied the penetration of bovine serum between negatively charged lecithin-cardiolipin mixed bilayers in a lamellar phase at pH 3.3, where the protein has a positive net-charge. BSA is also likely to adopt a more expanded structure at this pH, thus exposing more hydrophobic segments. He found that the inter-lamellar spacing of the lamellar phase, decreased with decreasing cardiolipin/bovine serum albumin ratio. This was related to a reduction of the negative charge of the lipid layer as the amount of bound protein increases. Bovine Serum Albumin, among other soluble proteins, was also found to increase the permeability of a fluorescent dye for negatively charged lipid vesicles but such effect was not observed for zwitterionic lipid vesicles (Ruggeri et al. 2013).

We will start our discussion by giving some example of the interplay between the lipid structures and protein in terms of the effect on the curvature of the lipid-aqueous interface, since curvature place an important role in condensed matter as discussed in the book by Hyde et al (1997).

5.3.3.1 Protein Interactions that Increase the Curvature of the Lipid-Aqueous Interfaces

Proteins or peptides that penetrate into the hydrophobic domain of a lipid bilayer generally provokes an increase of curvature of the lipid-aqueous interface, i.e. becomes more concave towards the aqueous space. Quite a few of the membrane bound peptides have these properties, such as Gramicidin A, a hydrophobic polypeptide, which forms channels for monovalent cations in phospholipid membranes (Wallis 1986). This peptide was found to favour the transition between lamellar phase → reversed hexagonal (H_{II}) phase in dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylethanolamine (DOPE) systems in an excess of water, as observed by NMR-studies (Chupin et al. 1987).

Not only proteins or peptides that penetrate into the lipid bilayer can induce phase transitions, but also proteins that mainly interact with the headgroups of the phospholipid bilayer can give rise to similar effects. This has been demonstrated for cytochrome c, which has a positive net-charge and has been shown to interact with negatively charged phospholipids (De Kruijff and Cullis 1980). The binding of cytochrome c to anionic cardiolipin liposomes induced the formation to an inverted hexagonal, H_{II}, structure (De Kruijff and Cullis 1980). No interaction and hence no phase transition was observed in the presence of liposomes composed of neutral zwitterionic lipids like PC and PE. A phase transition to the H_{II}-phase was observed, if a sufficient fraction of these lipids was replaced for cardiolipin. Interestingly, the protein was found to interact with liposomes of the anionic lipid phosphatidylserine

(PS), but did not induce any phase transition. The interaction between cardiolipin and cytochrome c was also studied by Spooner and Watts, using deuterium and phosphorus 31 NMR measurements (1991b). They likewise found that the interaction can cause a transition from a lamellar to a non-bilayer structure and that this depended on the lipid stoichiometry. The binding of the protein with the liquid-crystalline bilayers of cardiolipin was also found to cause extensive rearrangement of the cytochrome c secondary structure (Spooner and Watts 1991a, b).

Studies of the interaction between cytochrome c and suspensions of DMPG or admixtures of dioleoylglycerol (DOG) or DOPC with DOPG also showed that binding of cytochrome c could promote an increase in surface curvature of the lipid aggregates from a bilayer structure (Heimburg et al. 1991). This is deduced from NMR-data where an isotropic peak occurs in the presence of cytochrome c, indicating cubic lipid phases, small spherical vesicles or extended bilayers with high local curvature. The structure of cytochrome c was found to change on binding to the lipid, and two forms, depending on the lipid composition, were identified with resonance Raman measurements:

1. close to the native conformation in solution
2. unfolded with the heme crevice opened.

The changes in protein structure could be correlated with the curvature of the lipid bilayer as illustrated in Fig. 5.9 as the ratio between the unfolded (II) and native (I) cytochrome c (cyt c) in DOPC/DOG dispersions versus DOG mol%. The presence of DOG was found to induce spontaneous curvature in the DOPG lipid bilayer in the

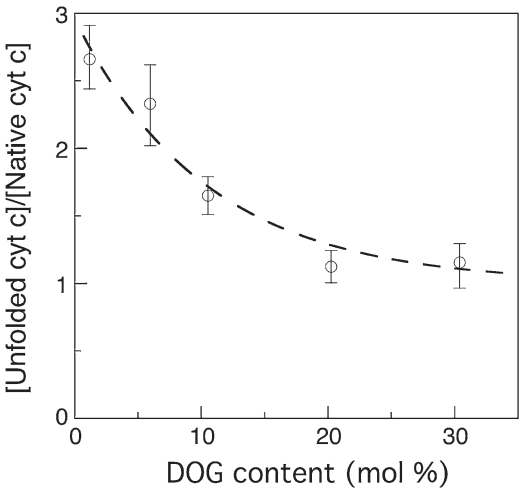

Fig. 5.9 Concentration of unfolded (II) and native (I) cytochrome c (cyt c) in dioleoylphosphatidylcholine (DOPC)/dioleoylglycerol (DOG) dispersions versus DOG mol% determined from Raman resonance spectra. The concentrations of lipid and cytochrome c were 300 and 20 μM, respectively, in an aqueous buffer (1 mM Hepes, 1 mM EDTA) of pH 7.5. Data adopted from Heimburg et al. (1991), where also the experimental details are given

pure lipid system, which at DOG content of ≈50% leads to the transition to a reversed hexagonal (H_{II}) phase. In the absence of DOG, that is a strict bilayer structure, the binding of the more unfolded form (II) of cytochrome is favoured, whereas the fraction of the more native globular protein structure (I) increases with the amount of DOG (Fig. 5.9) and thus with curvature of the surface. The physical state of the lipid was also found to affect the proportions of the two structural forms of cytochrome c. In the fluid state of pure DMPG, the fraction of the more unfolded form (II) was larger (85%) than when the lipid was in the gel state (80%). It is noteworthy that they found that the bound fraction of the more unfolded form (II) to the fluid DOPG bilayer structure was substantially lower (75%), indicating that not only the fluidity of the bilayer matters, but also the type of lipid.

The interaction between cytochrome c and monoolein in the cubic phase was studied by Razumas et al. by differential scanning calorimetry (DSC) and optical microscopy (Razumas et al. 1996b). In line with the studies reported above they also found that the presence of cytochrome c at high enough concentrations favored lipid aggregates with a larger curvature. Thus they observed that the phase transitions cubic → H_{II} → L_2 in the monoolein—cytochrome c—water system took place at a lower temperature than in the binary monoolein—water system (Razumas et al. 1996b). Similar effects were observed when glucose oxidase was included into monoolein-aqueous cubic phase (Barauskas et al. 2000). The temperature of the phase transition cubic → H_{II} in the monoolein—glucose oxidase aqueous system decreased with increasing glucose oxidase concentration.

It should also be noted that the interaction of lipolytic enzymes has more profound affects on the self-assembly structure. Due to their ability catalyze lipid hydrolyses, they will change the lipid composition and hence the packing parameter of the lipid followed by changes in the phase behavior (Borné et al. 2002a; Fong et al. 2014; Wadsater et al. 2014). This effect will be discussed further below.

5.3.3.2 Protein Interactions that Decrease the Curvature of the Lipid-Aqueous Interfaces

McCallum and Epand (1995) found that changing the curvature of biological membranes could modify membrane bound insulin receptor autophosphorylation and signaling. This was demonstrated by adding compounds that raised the bilayer to reverse hexagonal (H_{II}) transition temperature of model membranes, that is decrease the curvature of the membrane. This inhibited the insulin stimulation of the receptor phosphorylation.

Fraser et al. (1989) investigated the ability of a range of basic proteins and polylysine to convert a reversed hexagonal (H_{II}) phase, consisting of dioleoylphosphatidylethanolamine (DOPE) and mixtures of DOPE and phosphatidylserine (PS), to stable lamellar (L_α) phases at pH 9 where DOPE is anionic and at pH 7 when it is zwitterionic. The proteins investigated were all capable of binding to the H_{II} -phase at pH 9, but only myelin basic protein and polylysine did induce transition to the

L_α -phase. Lysozyme formed a new H_{II} -phase where the protein was included. A lowering of the pH seemed to release the proteins, except for mellittin, which also seemed to penetrate into the hydrophobic core of the lipid aggregates. The presence of PS in the H_{II} -phase at pH 7 increased the protein binding, but only interaction with myelin basic protein gave a lamellar phase. Based on earlier studies, Fraser et al. (1989) suggested that the myelin basic protein stabilized the lamellar phase by interacting with the DOPE head-group and thereby increasing its effective size. They concluded that the properties of myelin basic protein in terms of stabilizing the lamellar structure could be related to the role of the protein to stabilize the myelin sheath multilayers.

5.4 Interaction Between Protein and Surfactants or Polar Lipids at Interfaces

Defining different plausible scenarios and principles and defining simple models.

Interactions between proteins and surfactants at air/water and oil/water interfaces has attracted considerable study in recent years because the consequences of competitive adsorption of these two species at these interfaces can often strongly influence dispersion (foam or emulsion) stability against coalescence. The majority of proteins have high affinity for interfaces, which they saturate at comparatively low concentrations compared to low molecular weight (LMW) surfactants (Coke et al. 1990; Dickinsson and Woskett 1989). Thus, on a mole for mole basis at low concentrations, proteins reduce the surface tension to a greater extent than LMW surfactants. However, the opposite effect is observed at high concentrations, because at saturation coverage with LMW surfactants, the interfacial tension of the interface is usually lower than that achieved by proteins, and as a result, the latter molecules will be displaced from the interface. The region where the two different components coexist in the interfacial layer is of greatest interest, since it is in this region that will mostly affect the stability of the system towards coalescence.

The mechanisms by which proteins, polar lipids or mixtures of them stabilise emulsions and foams can be quite different. Generally, polar lipids are capable of reducing the interfacial tension more than proteins, while the protein molecules can be anchored at multiple sites at the interface. In principle, thin films are stabilized by two distinct mechanisms; the one that dominates is dependent upon the molecular composition at the interface (Clark 1995). Low molecular weight surfactants such as food emulsifiers or polar lipids congregate at the interface and form a fluid-adsorbed layer at temperatures above their transition temperature (see Fig. 5.10). When a surfactant-stabilized thin film is stretched, local thinning can occur in the thin film. This is accompanied by the generation of a surface-tension gradient across the locally thin region. Surface tension is highest at the thinnest point of the stretched film, due to the local decrease in the surface concentration of emulsifier in the region of the stretch. Equilibrium surface tension is restored by adsorption of surfactant

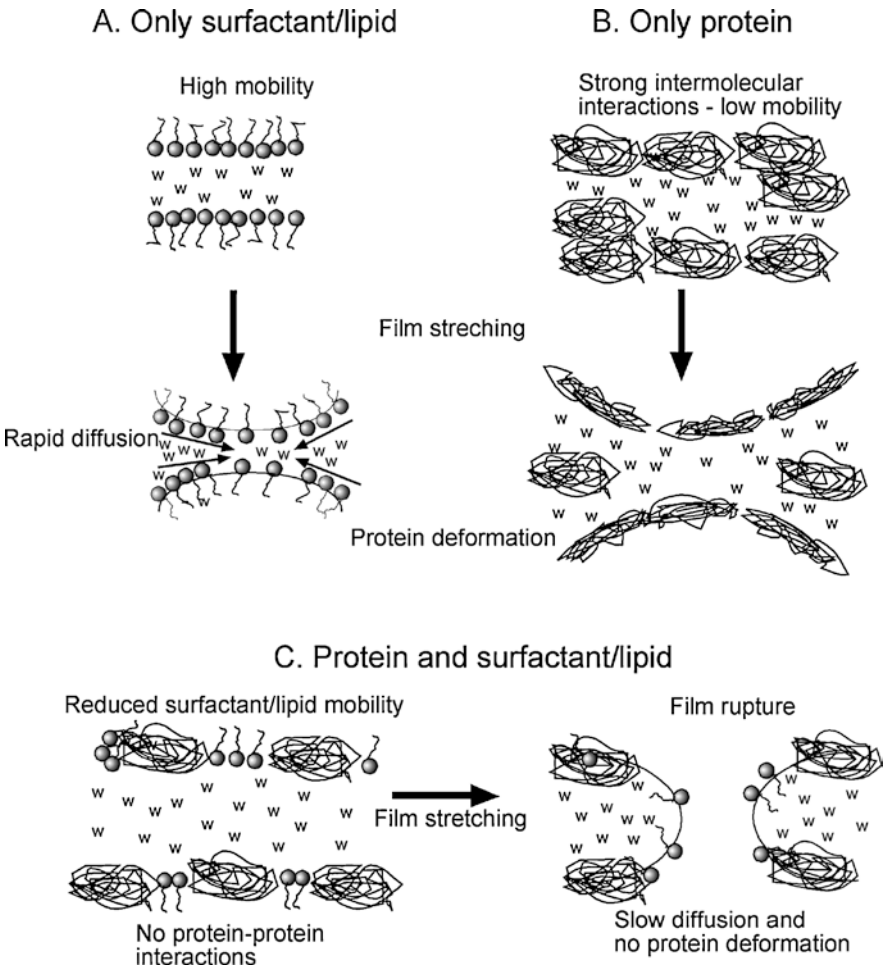

Fig. 5.10 The figure depicts possible mechanism for the stabilisation—destabilisation of foams with surfactants/lipids (**a**), proteins (**b**) and mixtures of the two components (**c**). Cross-sections of the thin films are shown where the aqueous inter-lamellar spacing is marked with (w). The stabilisation of the surfactant/lipid foams are based on the high lateral mobility of the surfactant, which makes it possible to quickly restore the surface tension gradient which arises from thinning of the film, i.e. the Gibbs-Marangoni effect. For protein stabilised foam the thinning is counteracted by strong intermolecular interactions which give a viscoelastic film. For the mixed system two mechanisms can counteract each other and leads to film rupture. The figure is adopted from Clark et al. (1991b)

from the interlamelIar liquid, which is of very limited volume in a drained thin film. This process is called the "Gibbs effect." Alternatively, migration of the surfactant by lateral diffusion in the adsorbed layer toward the region of highest surface tension may also occur (Clark et al. 1990). Here, the surfactant drags interlamellar liquid associated with the surfactant head group into the thin region of the film and

contributes to the restoration of equilibrium film thickness. This process is often referred to as the "Marangoni effect" (Ewers and Sutherland 1952).

In contrast, the adsorbed layer in protein-stabilized thin films is much stiffer and often has viscoelastic properties (Castle et al. 1987). These derive from the protein/protein interactions that form in the adsorbed layer (see Fig. 5.10b). These interactions result in the formation of a gel-like adsorbed layer, referred to as a "protein-skin"(Prins 1999), in which lateral diffusion of molecules in the adsorbed layer is inhibited (Clark et al. 1990). Multilayer formation can also occur and serves to further mechanically strengthen the adsorbed layer (Coke et al. 1990). When pure protein films are stretched, the change in interfacial area is dissipated across the film, due to the cohesive nature of the adsorbed protein layer and possibly the deformability of the adsorbed protein molecules.

Thin-film instability can result in systems that contain mixtures of proteins and low molecular weight surfactants (Clark et al. 1991a; Coke et al. 1990; Sarker et al. 1995), as is the case in many foods. The origin of this instability rests in the incompatibility of the two stabilization mechanisms: the Marangoni mechanism relying on lateral diffusion, and the viscoelastic mechanism on immobilization of the protein molecules that constitute the adsorbed layer. One can speculate that in a mixed system, competitive adsorption of low molecular weight surfactant could weaken or interfere with the formation of protein/protein interactions in the adsorbed layer and destroy the integrity and viscoelastic properties of the adsorbed layer (see Fig. 5.10c). This could be a progressive process, with the presence of small quantities of adsorbed surfactant initially introducing faults or weaknesses in the protein film. Adsorption of more surfactant could induce the formation of protein "islands" in the adsorbed layer. These structures could be capable of slow lateral diffusion but would be too large to participate in Marangoni-type stabilization. Indeed, they could impede surfactant migration in the adsorbed layer. Adsorption of progressively more surfactant would reduce the size of the protein aggregates still further until the adsorbed protein was in its monomeric form. Ultimately, all the protein would be displaced from the interface by the surfactant.

Two types of interaction are shown in the schematic diagram of the mixed system. First, there is an interactive process associated with the co-adsorption or competitive adsorption of the two different species at the interface. Second, many of the functional proteins used in food production have physiological transport roles and therefore possess binding sites, which may allow the formation of complexes with surfactants.

A clearer understanding of this has emerged from direct study of the structures that separate the dispersed-phase of foams or emulsions, under conditions of high dispersed phase volume (i.e., foam or emulsion thin films). Such structures form rapidly in foams following limited drainage but may occur only in emulsions after creaming of the dispersed phase.

Several factors control the emulsifier-protein interaction at the interface.

On the bases of experimental data the following factors influence the way mixtures of proteins and emulsifiers, eg. surfactants and polar lipids behave at an interface:

1. The surface activity of the individual components.
   (a) Competitive adsorption.
   The emulsifier and proteins compete for the interface, where the most surface active and/or abundant molecule wins, depending on the ratio between surfactants and proteins in solution.
   (b) Displacement.
   The emulsifier may, due to their higher surface activity, displace the proteins from the interface. This displacement can be hampered by a strong interaction between the protein and the interface and/or protein-protein interactions.
2. Protein-emulsifier interactions.
   (a) Increased surface activity of the emulsifier-protein complex
   - The binding will cause unfolding and/or increase hydrophobicity of the protein that will lead to an increased affinity to the surface.
   - The binding (of ionic amphiphiles) will cause precipitation at the interface due to charge neutralisation.

   (b) Decreased surface activity of the emulsifier-protein complex
   - The binding will make the protein more soluble and hence lower the affinity for the interface.
   - The binding will lead to precipitation of protein lipid-complex in the bulk, which will cause loss of surface-active material.

   (c) Protein-emulsifier interactions at the interface
   - The interaction will give more efficient packing at the interface and thus give a higher total surface concentration.
   - The interaction will disrupt the protein-protein interaction in the interfacial film.

It is important to bear in mind that different modes of interaction are observed for the same system depending on the emulsifier/protein ratio. This can be manifested in the competitive adsorption of emulsifier and proteins. Studies regarding such surfactant/protein "Vroman effects[2]"have been reported; for example, adsorption of fibrinogen from mixtures containing Triton X-IOO passes through a maximum (Slack and Horbett 1988). Wahlgren and Arnebrant (1992) studied the adsorption from β-lactoglobulin/SDS mixtures at different degrees of dilution (see Fig. 5.11). At concentrations above the cmc for the surfactant, the amount adsorbed corresponded to a layer of pure surfactant and was found to increase after rinsing. At lower concentrations, the adsorbate prior to rinsing appeared to be a mixture of

[2] The "Vroman effect" is the hierarchical adsorption process of blood protein, where the first proteins to be adsorbed are the relatively abundant plasma proteins, such as albumin, fibrinogen, immunoglobulin G and fibronectin, which are soon replaced by trace proteins, including factor XII (Hageman factor) and high molecular weight kininogen (HMWK) with higher affinity to the surface (Brash and Hove 1984; Horbett 1984; Vroman et al. 1980).

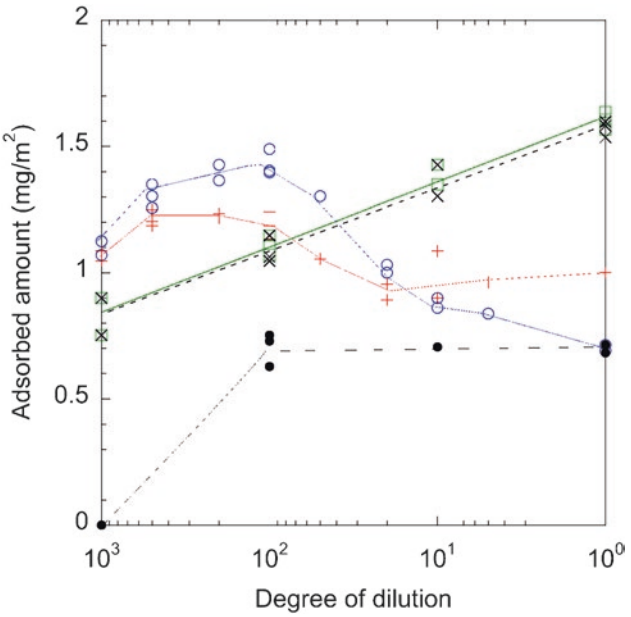

Fig. 5.11 The amounts adsorbed to a methylated silica surface as a function of degree of dilution for a mixture of β-lactoglobulin and SDS (0.2 w/w), in phosphate buffered saline pH 7, I = 0.17. The figure shows the adsorbed amount (μg/cm^2) after 30 min of adsorption (○-Blue) and 30 min after rinsing (+). In addition, the figure shows the adsorption of pure β-lactoglobulin, after 30 min of adsorption (□-Green) and 30 min after rinsing (x). Finally, the adsorption isotherm of SDS is inserted (●). (From Wahlgren and Arnebrant 1992)

protein and surfactant, and the total amount adsorbed passes through a maximum. The amount of protein adsorbed is larger, even after rinsing, than for adsorption from pure β-lactoglobulin solutions, and thus it can be concluded that SDS binding in this case facilitates the adsorption of protein.

5.4.1 Influence of Emulsifier Properties

The emulsifier properties affect the interaction with proteins and surfaces as well as the structure of the formed self-assembled aggregate.

5.4.1.1 Aqueous Soluble –Surfactant Type of Emulsifiers

Wahlgren and coworkers studied the influence of different surfactant head groups on the desorption of adsorbed lysozyme (Wahlgren and Arnebrant 1991, 1992; Wahlgren et al. 1993a) by surfactants at concentrations above the cmc (an exception

was triethylene glycol n-dodecyl ether, [$C_{12}E_3$], which does not form micelles (Mitchell et al. 1983). The difference between the effect of sodium dodecylsulphate (SDS) and cationic and nonionic surfactants on protein adsorption to hydrophilic surfaces was found to correlate to the strength of binding to protein in solution. This suggests that above the critical association concentration (cac), complex formation between surfactant and protein is involved in the removal mechanism of proteins from hydrophilic solid surfaces. In the case of hydrophobic solid surfaces, the removal processes of protein by the different surfactants, including non-micelle-forming ones, are in general more similar than for the hydrophilic surfaces. This might be expected, due to the different orientation of the surfactant, and suggests a displacement mechanism, due to higher surface activity of the surfactant (Wahlgren and Arnebrant 1992). Tilton and coworkers used the interferometric surface force technique (Israelachvili and Adams 1978) to study the interaction between lysozyme adsorbed on mica and SDSo (sodium dodecane sulfonate) and SDS (Tilton et al. 1993). They found that SDSo, which has a Krafft temperature above room temperature and hence does not form micelles, had a minor effect on the interaction between adsorbed lysozyme layers on mica, and from the small change in surface potential, they concluded that few surfactant molecules were bound to the adsorbed protein. SDS showed a similar low binding to lysozyme on mica at low concentrations (up to 0.5 cmc) but caused a collective desorption of the protein at the cmc of the surfactant, indicating that the cac to adsorbed lysozyme is in the range of its self-association limit in solution (cmc) (Froberg et al. 1999). These studies show that anionic surfactants bind to an adsorbed layer of lysozyme, which is almost neutral after binding of the positively net-charged protein to the negative mica surface. The binding of surfactant thus leads to an increased negative charge of the layer, which in the case of SDS finally leads to desorption of the protein. It is likely that this is due to electrostatic repulsion between the negatively charged surface and the protein/surfactant complexes.

Nonionic surfactants are generally found to be ineffective in removing protein from hydrophilic solid surfaces (Welin-Klintström et al. 1993; Elwing and Golander 1990; Elwing et al. 1989). As mentioned above, these surfactants bind to a very low extent to protein in solution (except when specific binding sites or pockets are present or in the cooperative regime, see Sect. 5.3.1.2.2) and to the protein-covered surface. At hydrophobic surfaces, however, they usually have a considerable effect (Wahlgren and Arnebrant 1996; Wannerberger et al. 1996) and could indeed completely block protein adsorption (Kapp et al. 2015). This was elegantly demonstrated in a study of surfactant interactions with proteins adsorbed at a surface with a gradient in wettability (Elwing et al. 1989).

The effect of chain length of alkyltrimethylammonium surfactants on the elutability of fibrinogen at concentrations above their cmc was found to be small at both silica and methylated silica surfaces (Wahlgren et al. 1993b). Rapoza and Horbett (1990b) did not find any effects of chain length of sodium alkyl sulfates on the elutability of fibrinogen and albumin down to a chain length of six carbon atoms. However, they found that the chain length did influence the surfactant concentration at which the onset of protein removal was initiated as expected. The trend was

similar to the one observed for the onset of other cooperative binding events (e.g., micelle formation).

Rapoza and Horbett (1990a) found that surfactants with large head groups such as Tween 20 gave lower fibrinogen elutability levels than other surfactants at polyethylene surfaces. Welin-Klintström et al. (1993) found that the elutability of fibrinogen adsorbed at surfaces with a wettability gradient decreased with the bulkiness of the hydrophobic part of the surfactant. In this connection it was also found that nonionic surfactants showed an increased removal of fibrinogen into the more hydrophilic region of the gradient surface when the cloud point (phase separation temperature) was approached (Wahlgren et al. 1995). These general observations of removal efficiency are in line with the findings from studies of the removal of fat by different surfactants (Backstrom et al. 1988; Malmsten and Lindman 1989), where a maximum removal was achieved at conditions corresponding to an optimum in the packing of surfactant molecules at a flat interface.

Thus, it may be concluded that at high surfactant concentrations, head group effects are, as expected, most pronounced at hydrophilic surfaces but less important at hydrophobic ones. In addition, it appears that principles for detergency in general, involving the packing efficiency of molecules at interfaces, are applicable to qualitatively describe the removal of proteins from the surface.

5.4.1.2 Lipids with Low Aqueous Solubility

5.4.1.2.1 Electrostatics

Phospholipid—β-lactoglobulin interactions at the air—aqueous interface have been investigated by Bos and Nylander (1995) using the surface film balance. Some of their findings are summarised in Fig. 5.12, where the rate of incorporation of β-lactoglobulin into monolayers of distearoylphosphatidic acid (DSPA), distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidic acid (DPPA) is shown versus surface pressure (Π) at pH 7. The rate was calculated using a simple first order kinetics model (MacRitchie 1990), where only the surface pressure barrier is taken into account. The highest rate of adsorption of β-lactoglobulin into a phospholipid monolayer was observed for anionic DSPA. The incorporation of the protein takes also place at a higher surface pressure into a DSPA monolayer than into monolayers of the other lipids. Since the β-lactoglobulin, with a zero net charge at pH ≈ 5.2 (Hambling et al. 1992), has a positive net-charged at pH 4, a larger rate of adsorption into the negatively charged phosphatidic acid monolayers would be expected under acidic conditions. However, almost the same rates were found (Bos and Nylander 1995). As discussed earlier, anionic lipids seems to interact more strongly with proteins, that is to cationic amino acid residues, compared to lipids with none or positive net-charge. The incorporation into the zwitterionic DSPC monolayers is as expected less salt dependent than what was observed for the phosphatidic acid monolayers, where the rate increases with increasing ionic

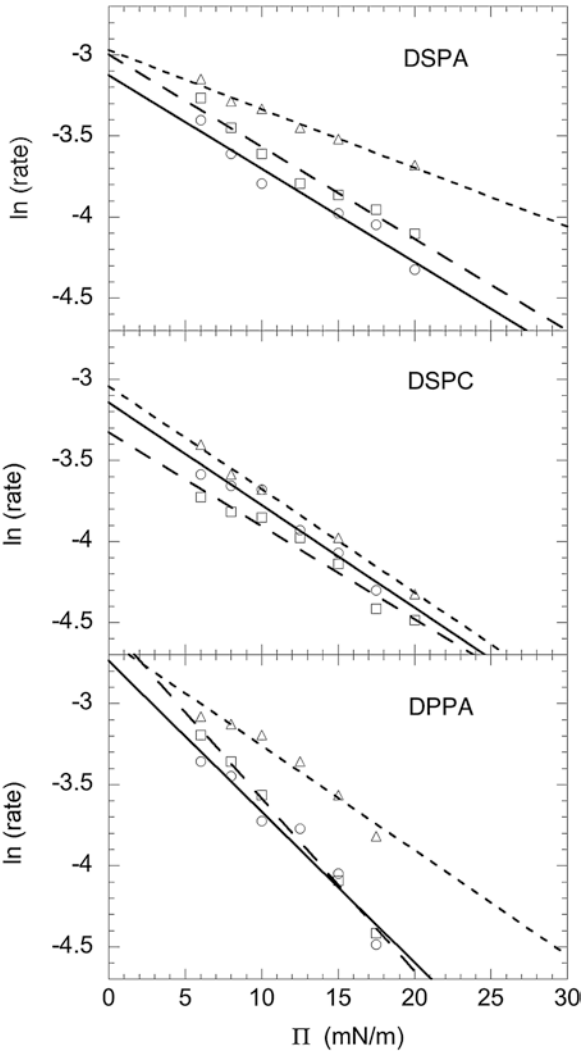

Fig. 5.12 The rate of incorporation of β-lactoglobulin into monolayers of distearoylphosphatidic acid (DSPA), distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidic acid (DPPA), versus surface pressure (Π). The data was recorded at constant surface pressure by measuring the area increase of the lipid monolayer spread on a protein solution contain 1.15 mg/l in 10 mM phosphate buffer of pH 7, with 0 mM (——○——), 50 mM (— — □— —) or 150 mM (- - - Δ- - -) sodium chloride. The rate in mg/m^2 was calculated from the area increase by using the Π-area isotherm of spread monolayers of β-lactoglobulin. Data adopted from Bos and Nylander (1995), where also the experimental details are given

strength of the subphase. Probably this is a consequence of a decreased repulsion within the phosphatidic acid protein monolayer at a higher ionic strength. The findings by Bos and Nylander (1995) is somewhat contradictory to the findings of Cornell and Patterson, who studied the adsorption of β-lactoglobulin into a negatively charged lipid monolayer, composed of a mixture of palmitoyloleoylphosphatidylcholine (POPC) and palmitoyloleoylphosphatidylglycerol (POPG) (65/35 mol%). They only observed a substantial binding of β-Lactoglobulin at pH 4.4, which is when the protein carries a net positive charge, but not at higher pH (pH 7) (Cornell and Patterson 1989). The differences probably arise from the use of different lipids and methodology used by Cornell et al. (Cornell 1982; Cornell and Patterson 1989; Cornell et al. 1990). Cornell et al. measured the amounts of protein adsorbed to the lipid layer by transferring the layer to a solid support. During the transfer, the surface pressure was kept at 30–35 mN/m, thus preventing insertion of portions of the protein in the lipid monolayer (Cornell et al. 1990). Only protein molecules that strongly attracted to the lipid headgroups are transferred to the solid supported. Another difference is that their surface pressure data of the protein penetration is recorded under constant area, not at constant pressure as in our study. In addition Cornell et al used lipids with their chains in the liquid state, which, as discussed below, can influence the interaction. Cornell (1982) also observed a specific interaction between β-lactoglobulin and egg yolk phosphatidic acid (e-PA) in spread mixed films at low pH (1.3 and 4) where β-lactoglobulin carries a positive net charge. No interaction was observed for e-PA in the neutral pH range or for egg yolk phosphatidylcholine, e-PC. Similar observations were made for the interaction between α-lactalbumin or BSA with mixed monolayers of POPC and POPG, where adsorption was observed below the isoelectric point of the protein where the lipid layer and the protein carry opposite net charge, but less was adsorbed around and almost nothing above the isoelectric point (Cornell et al. 1990). The interaction was reduced in the presence of calcium as well as at increased ionic strength. Cornell et al. thus concluded that the interaction is of electrostatic origin.

The work of Quinn and Dawson (1969a, b) concerning the interaction between cytochrome c (positive net charge below pH 10) and phospholipids from egg yolk also stresses the importance of the electrostatic interaction, although conformational changes of the protein are of importance. They measured the pressure increase caused by the penetration/adsorption of the protein to the lipid monolayers as well as the amount adsorbed by using ^{14}C-labeled protein. Their results show that the limiting pressure for penetration is 20 and 24 mN/m for phosphatidylcholine and phosphatidylethanolamine, respectively, whereas penetration into the phosphatidic acid and diphosphatidylglycerol (cardiolipin) monolayers occurred up to pressures close to the collapse pressure of the film (<40 mN/m). Furthermore, the penetration into the e-PC monolayers was not affected by increasing the sodium chloride concentration to 1 M. Cytochrome c bound to the e-PC monolayers could not be removed by increasing the ionic strength. This is in contrast to the cardiolipin and e-PA monolayers where the penetration was reduced when the sodium chloride content was increased to 1 M. It was also possible to partly desorb some cytochrome c from e-PA monolayers. However, the pH dependence of the interaction was found

to be quite complex, which suggests that subtle changes in the protein conformation also affect the interaction.

The importance of the electrostatic interaction with the phospholipid head group has also been shown by the work of Malmsten (Malmsten et al. 1994; Malmsten 1995), who studied the interaction of human serum albumin, IgG and fibronectin from human plasma with phospholipid layers spin-coated onto methylated silica surfaces. Generally, no interactions between the proteins and lipids with no net charge or with shielded charges (e.g. phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and phosphatidylinositol) took place, whereas interaction was observed with the surfaces containing unprotected charges, e.g. phosphatidic acid, diphosphatidylglycerol and phosphatidylserine.

5.4.1.2.2 Hydrophobic Interactions

As observed in Fig. 5.12 the rate of adsorption of β-lactoglobulin into DPPA monolayers was significantly lower than into the monolayers where the corresponding lipid had a longer chain length. This points to the importance of hydrophobic interactions for the incorporation. It was also observed that the incorporation was much faster into the lipid monolayer than into its own proteinaceous layer, being less "oil-like" than the lipid layer (Bos and Nylander 1995). In addition, repulsive steric and electrostatic forces might contribute the lower rate of incorporation. Quinn and Dawson (1969b) found that the threshold surface pressure, above which no penetration of cytochrome c took place in phosphatidylcholine monolayers, was considerably lower when DPPA was used instead of hydrogenated egg yolk phosphatidylcholine (e-PC). The latter lipid contained fatty acid with a longer chain length, about 60% C18 and 30% C16. Du et al. (1996) studied the influence of the alkyl chain length of glycolipids (dialkyl glycerylether-β-D-glucosides and dialkyl glycerylether-β-D-maltosides) on the interaction between lipid monolayers and glucose oxidase. The interaction, as shown by an increase in surface pressure, was found to increase with increasing lipid chain lengths for both types of lipids. These results suggest that the hydrophobic interaction is the predominant force. Furthermore it is interesting to note that the interactions were not so strong with the lipids having the more bulky head group, that is the dialkyl glycerylether-β-D-maltosides, although the Π-A isotherms for the corresponding dialkyl glycerylether-β-D-glucosides was similar. This illustrates that a bulky head group can sterically hamper the protein-lipid (hydrophobic) interaction.

5.4.1.2.3 Effect of Lipid Fluidity

The complete hydrogenation of e-PC was found not to affect the surface pressure threshold for penetration of cytochrome c compared to the native e-PC (Quinn and Dawson 1969b). However, the change in surface pressure due to the penetration of the protein versus initial surface pressure was less steep for the saturated one. A

similar trend was observed for the e-PE samples (Quinn and Dawson 1969a). The conclusion was that the limiting pressure for penetration to take place is likely to be determined by the work necessary for the penetration, that is $\int \Pi dA$, where an area of interface, A, has to be created for the protein to penetrate. Once the penetration is feasible the magnitude will depend on the space between the molecules and thus the degree of penetration is expected to be lower for the hydrogenated sample (Quinn and Dawson 1969b). The surface pressure threshold below which penetration of cytochrome c into the anionic diphosphatidylglycerol (cardiolipin) monolayer took place was also found to decrease when the lipid was fully hydrogenated (Quinn and Dawson 1969b). Ibdah and Phillips (1988) found the same trend in their study of the effect of lipid composition and packing on penetration of apolipoprotein A–I into lipid monolayers. In the biological system this protein interacts with the phospholipid membrane of the serum high density lipoprotein (HDL) particles (see discussion in oil/aqueous interfaces, Sect. 5.4.3). Their results show that for this protein, adsorption occurs to a larger extent on expanded monolayers than on condensed monolayers, that is, protein adsorption decreased in the order e-PC > egg sphingomyelin > DSPC. Furthermore it was found that protein adsorption generally decreased with increasing amount of cholesterol in the lipid monolayer. It was suggested this was due to the condensing effect of cholesterol.

5.4.1.3 Other Types of Surfactants

Blomqvist et al. (2004, 2006) investigated the effect of the poly(ethylene oxide)-poly(propylene oxide) block copolymers F127 (PEO99-PPO65-PEO99), molecular weight 12,500 g/mol, and P85 (PEO26-PPO39-PEO26), molecular weight 4600 g/mol on β-lactoglobulin foamability and foam stability. They found that the effect of the nonionic triblock copolymer on the interfacial rheology of β-lactoglobulin layers is similar to that of low molecular weight surfactants (Blomqvist et al. 2004). However the protein foam stability was retained in the presence of the larger polymer F127, whereas P85 largely reduced the stability (Blomqvist et al. 2006). This shows that here the size of the amphiphilic polymer has a significant effect. The presence of F127 was found to increase thickness of the foam lamellae, which in turn reflects the increased steric repulsion.

5.4.2 Influence of Protein and Protein Film Structure

The stability of the proteins largely affects the interaction with the emulsifier and the interface. Differences are observed between the random coil and globular proteins. The age of the surface layer of proteins that tend to aggregate can significantly decrease the penetration of the emulsifier in the surface layer.

Even though ionic surfactants may interact, more or less specifically with charged residues of proteins, especially at low concentrations (see Sect. 5.3), no clear

relation could be established regarding the influence of protein net charge on the interaction with ionic surfactants at high surfactant concentration (McGuire et al. 1995a; Wahlgren and Arnebrant 1991; Wahlgren et al. 1993a). This might, of course, be related to the fact that in principle all proteins contain both negative and positive charges except at extreme pH. In an effort to determine key protein parameters for their interaction with surfactants, Wahlgren and coworkers (1993a) studied the DTAB-induced removal of six adsorbed proteins: cytochrome c, bovine serum albumin, α-lactalbumin, β-lactoglobulin, lysozyme, and ovalbumin from silica and methylated silica surfaces. For silica surfaces, it was found that the removal of the proteins that were still adsorbed after rinsing with buffer, increased with decreasing molecular weight, adiabatic compressibility [a measure of conformational stability (Gekko and Hasegawa 1986)] and increasing thermal denaturation temperature (Wahlgren et al. 1993a). In the case of hydrophobic (methylated silica) surfaces, differences between the proteins were smaller. However, increasing molecular weight and shell hydrophobicity of the protein seemed to reduce the degree of removal. Moreover, the removal did not relate to the degree of desorption of proteins upon rinsing with buffer, indicating that the mechanisms for the two processes are different. McGuire et al. (1995b) found that the removal of wild type and structural stability mutants of bacteriophage T4 lysozyme from hydrophobic and hydrophilic silica surfaces by a cationic detergent, decyltrimethylammonium bromide (DTAB), generally increased with the stability of the mutants.

The effect of the interfacial protein film age on the displacement of the protein from the surface of emulsion drops by nonionic water soluble surfactants (Tween 20 and octaethylene glycol *n*-dodecyl ether ($C_{12}E_8$)) showed that β-lactoglobulin is harder to replace the longer the residence time was (Chen and Dickinson 1993; Chen et al. 1993). Similar results were obtained for a range of other protein (Bohnert and Horbett 1986; Rapoza and Horbett 1990a). Apart from the possible conformational changes that occur during the adsorption process, which can hamper displacement, it has been reported that β-lactoglobulin might polymerise through disulphide exchange at the oil-water interface (Dickinson and Matsumura 1991). Consequently, the displacement of β-casein, which is a flexible and unordered protein without sulfhydryl groups, did not depend on the age of the film. Furthermore, it was harder to replace β-lactoglobulin from a emulsion prepared close to the pI of the protein, than at neutral pH, whereas the replacement from emulsions prepared at pH 3 was easier and effect of the age of the protein film was observed. Mackie et al. (1999) also studied displacement of β-lactoglobulin and β-casein by Tween 20, but from the air-water interface. They also found that β-casein was more easily displaced, i.e. β-lactoglobulin films breaks at higher surface pressures. Stress invoked by penetration of the surfactant was found to propagate homogenously through the β-casein film, which in turn resulted in growth of circular surfactant domains at the interface. β-Lactoglobulin, on the other hand was found to form elastic (gel-like) networks at the air-water interface and the penetration of the surfactant therefore resulted in the growth of irregular (fractal) surfactant domains. Not surprisingly, Tween 20 preferentially displaced β-casein before β-lactoglobulin

from a mixed β-casein/β-lactoglobulin film at the air-water interface (Mackie et al. 2001b).

5.4.3 Influence of Surface Properties

The surface properties affect the binding of the emulsifier as well as of the protein and has therefore large effect on the competitive adsorption.

The surface activity of the complex depends on the properties of the interface, as shown by Wilde et al. (Wilde and Clark 1993) for liquid interfaces. They found that the complex between Tween 20 and β-lactoglobulin was more surface active at the oil-water interface than at the air-water interface, where the same surface activity as for the free (or pure) protein was observed. The complexes adsorbed at both type of interfaces were however displaced by Tween 20 at the same surfactant to protein ratio. Here, we need to emphasize the difference in nature between the two types of liquid interfaces, the liquid/air and the one between two condensed media, which explains the experimental observations. The oil/water interface allows hydrophobic residues to become dissolved in and interact favorably with the oil phase, which is not possible at the air/water interface. We have also previously discussed that the unfolding of protein induced by the action of surfactants or by the presence of an interface generally leads to exposure of hydrophobic residues, that is the unfolded protein can be substantially more "oil soluble" than the native one. This relates to the following section, dealing with molecular interactions, where it will be demonstrated, that changes in oil phase composition and hence solvent properties, also can lead to changes in the structure of the adsorbed protein film.

5.4.3.1 Solid-Liquid Interfaces (Dispersions and Macroscopic Surfaces)

Protein/surfactant interactions at solid-liquid interfaces have been studied with the aim of estimating the protein attachment strength to surfaces, for optimizing detergency processes, and for avoiding undesired adsorption in biomedical applications. The major part of the work has been carried out with the purpose of characterizing the protein binding to the surface rather than the protein/surfactant interaction and therefore concerned with the degree of removal, or elution, of adsorbed protein by surfactant. Even if the data mainly refer to solid surfaces, the basic principles are also valid at liquid interfaces such as those of the emulsion droplet. Since the process of surfactant interaction with proteins at interfaces is determined by the surfactant/protein, the surfactant/surface and protein/surface interactions, the following brief introduction is intended to provide a background on surfactant association and adsorption,

The adsorption and orientation of surfactants are dependent on the type of surface. There is a vast literature concerning the association of surfactants at solid/

aqueous interfaces (Manne et al. 1994; Scamehorn et al. 1982; Zhang and Somasundaran 2006; Zhmud and Tiberg 2005). The structure of the surface aggregates at the plateau has been debated, and surface micelles, finite bilayers, or infinite bilayers have been suggested for hydrophilic surfaces. It has been demonstrated that non-ionic polyethylene glycol monoalkyl ethers (C_nE_m) adsorb as submonolayers or monolayers on hydrophobic surfaces, while they form surface micelles or bilayer type aggregates (depending on the type of surfactant) on hydrophilic surface (Tiberg 1996) Fig. 5.13. It is therefore natural to expect that the way in which surfactants interact with proteins should be influenced by the characteristics of the surface as well.

Wahlgren and Arnebrant (1991) investigated the effect of the surface properties on the displacement of adsorbed β-lactoglobulin (negative net charge) and lysozyme (positive net charge) by the cationic surfactant cetyltrimethyl ammonium bromide (CTAB) and the anionic sodium dodecyl sulphate (SDS). They used hydrophobic (hydrophobised silica), negative (hydrophilic silica), neutral (chromium oxide) as well as positively charged (nickel oxide) surfaces and found four types of behavior for surfactant concentrations well above cmc:

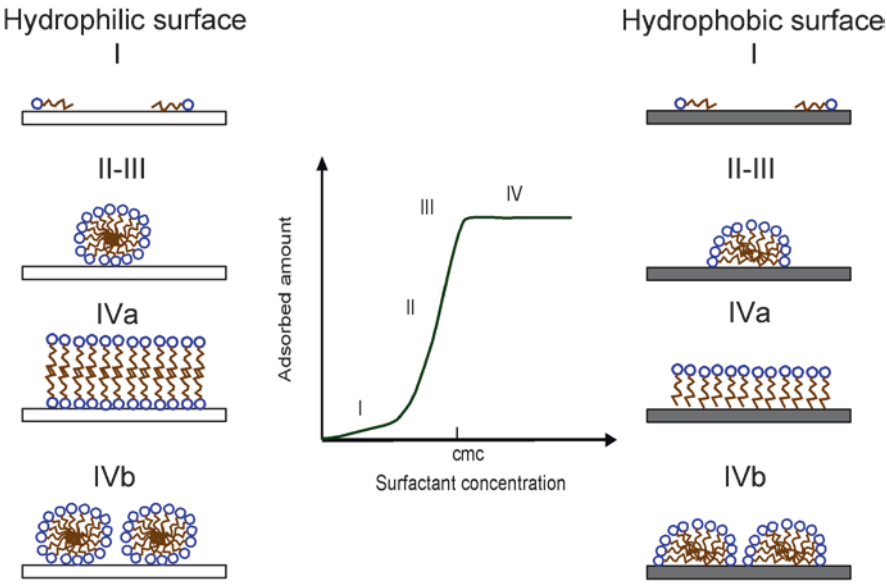

Fig. 5.13 An illustration of probable arrangements of adsorbed surfactant molecules at different degrees of surface coverage. Adsorption to hydrophilic surfaces (upper panels) and hydrophobic ones (lower panels). The illustrations are drawn to represent structures having minimal water contact with the hydrophobic parts of the molecules. The labels (I) to (IV) refer to structures that may exist in different regions of the isotherm. The figures should be considered as schematic and other structures, especially for ii to iii, have been suggested

1. Surfactant binds to the protein and the complex desorbs on dilution.
   This was observed for SDS and β-lactoglobulin as well as lysozyme on negative silica surface and can be explained by simple electrostatic considerations. No adsorption from SDS/protein mixtures occurred.
2. The surfactant replaces the protein at the interfaces.
   This requires that the surfactant interacts more strongly with the surface than the protein, as was observed for CTAB with negative silica and SDS and CTAB with the hydrophobic surface when the adsorbed layer consisted of β-lactoglobulin. This can also occur for non-ionic surfactants well above the cmc (Kapp et al. 2015).
3. The surfactant co-adsorbs reversibly to the protein layer.
   The protein surface interaction is the stronger one and the surfactant is thus unable to solubilize the protein from the interface. This was observed for CTAB interacting with both proteins at the chromium oxide surface and SDS interacting with β-lactoglobulin at the nickel oxide surface. This can also occur for non-ionic surfactants below the CMC (Kapp et al. 2015).
4. Partial removal of the protein.
   This can be explained as due to the presence of multiple binding sites for the protein, and can result from either mechanism 1 and 2.

5.4.3.1.1 Surface Charge

One can imagine several ways that emulsifiers can modulate the interaction of proteins with the surface depending on the charge of the surfactant, protein net-charge and the surface charge. Here it is important to point out that ionic emulsifiers can affect the amount of protein on the surface by modifying the protein-surface interaction by changing the surface charge and/or protein charge as well as the interaction between adsorbed protein/emulsifier interaction.

Green et al. (2001) studied the interaction between sodium dodecyl sulfate (SDS) and preadsorbed lysozyme at the hydrophilic silicon oxide-water interface by neutron reflectivity measurements. SDS binds cooperatively to the preadsorbed protein layer at intermediate surfactant concentrations, with no desorption of lysozyme from the interface. The protein was partly removed when the SDS concentration was increased to above 0.5 mM while 2 mM SDS was required to completely remove both protein and surfactant from the interface. The surfactant-protein complex and the surface is then likely to both be negatively charged and the electrostatic interaction cause desorption.

Indirectly the neutron reflectivity study on the binding of SDS onto preadsorbed layers of bovine serum albumin (BSA) at the hydrophilic silicon oxide-water interface by Lu et al. (1998) confirm the "orogenic" displacement model (Mackie et al. 1999, 2001b, b) discussed above. The specular neutron reflection is sensitive to the density profile normal to the interface, but does not give any lateral resolution. Their results suggest uniform layer distribution of SDS at low surfactant

concentrations, while the distribution becomes unsymmetrical as the SDS concentration increases. The binding of SDS results in an expansion of the preadsorbed BSA layer from 35 Å in the absence of SDS to some 80 Å at 3×10^{-4} M SDS, which Lu et al. interpreted as a considerable structural deformation of the protein. They based this interpretation on the close agreement between the volume ratio of SDS to BSA in the mixed layer of 0.45, and the literature value for the binding of SDS onto denatured protein in the bulk reported by Tanner et al. (1982).

Investigations into the elutability of lysozyme and β-lactoglobulin from methylated silica (hydrophobic) and oxides of silicon, chromium, and nickel by SDS and cetyl trimethyl ammonium bromide (CTAB) showed no simple correlation between the fraction removed and the difference between the two oppositely charged surfactants. Instead, elutability of β-lactoglobulin and lysozyme decreased roughly in the order silica > chromium oxide > nickel oxide (Wahlgren and Arnebrant 1991). In these cases the extent to which the protein is removed mainly reflects the binding mode of the protein to the surface.

5.4.3.1.2 Surface Hydrophobicity

Elwing et al. (Elwing et al. 1989; Elwing and Golander 1990) studied the surfactant elutability of proteins adsorbed to a surface containing a gradient in hydrophobicity and found large differences in the amounts removed from the hydrophilic and hydrophobic ends. In the case of a nonionic surfactant (Tween 20), the elutability was largest at the midpoint of the gradient, which can be attributed to enhanced conformational changes of the adsorbed protein at the hydrophobic end, in combination with a lower efficiency of removal by nonionics at hydrophilic surfaces. At hydrophobic surfaces the removal is generally high (Elwing et al. 1989; Wannerberger et al. 1996). However, this may not be considered as evidence for weak binding of the proteins to the surface, but rather as an indication of the strong interaction between the surfactants and surface.

Horbett and coworkers (Bohnert and Horbett 1986; Rapoza and Horbett 1990a) studied the elutability of fibrinogen and albumin at different polymeric surfaces and found that the elutability and the change in elutability with time differed between surfaces. These differences could not, however, be correlated to surface energy in terms of their critical surface tension of wetting.

5.4.3.2 Liquid-Liquid Interfaces (Emulsions and Vesicles)

Most studies of protein-lipid interactions at the oil aqueous interface have been carried out using model emulsions. The purity of polar lipid and the way it is added (e.g. to the oil or the water phase) are bound to affect the interactions with proteins, which in turn affect the emulsion stability. Yamamoto and Araki (1997) studied this by comparing the interfacial behavior of β-lactoglobulin, in the presence of lecithin (PC) in the water or in the oil phase, with the stability of corresponding emulsions.

In the presence of protein, crude lecithin was found to increase the stability of emulsion and lower the interfacial tension more effectively than a pure lecithin preparation. When crude lecithin was added to the oil phase the interfacial tension was found to decrease, and the emulsion stability increased as compared to when the lecithin was dispersed in the aqueous phase. One might speculate if these findings can be related to the presence of fatty acid and/or charged phospholipids in the crude lecithin. Aynié et al. studied the interaction between nitroxide homologues of fatty acids and milk proteins by following the mobility of the nitroxide radicals using electron spin resonance (1992). At pH 7, the importance of the lipid protein interaction was not determined by the structure of the protein, but positively correlated with the number of positive charges on the protein. Thus, it was observed that the importance of the interaction in the emulsions decreased in the order α_{s1}-casein > β-lactoglobulin > β-casein, suggesting that the interaction was of electrostatic nature. The different proteins also affect the organisation of lipid monolayer, where α_{s1}-casein in contrast to β-lactoglobulin and β-casein, induce an ordering of a monolayer of nitroxide fatty acids on the surface of an emulsion droplet (Aynié et al. 1992). This can probably be assigned to the stronger interaction of α_{s1}-casein with lipids compared to the other proteins.

Bylaite et al. (2001) applied ellipsometry to study the adsorption of the lipid from the oil and the protein from the aqueous phase at the oil-water interface. Independently of the used concentration, close to monolayer coverage of soy bean PC (sb-PC)) was observed at the caraway oil—aqueous interface. On the other hand, at the olive oil—aqueous interface, the presence of only a small amount of sb-PC lead to an exponential increase of the layer thickness with time beyond monolayer coverage. This interesting observation was assigned to the formation of a multilamellar layer of sb-PC at the olive oil—aqueous interface, when sb-PC reached the solubility limit in the olive oil. The properties of the interfacial phase at a given time were found to depend strongly on whether phospholipid was added to the oil phase or to the aqueous phase as liposomal structures (Benjamins et al. 2005). In the latter case a monolayer formed, while if the phospholipid was supplied from the oil phase a lamellar phase appeared at the interface. The kinetics of the processes differs: Monolayer coverage from the liposomal dispersion is a rapid process, while the formation of the intermediate lamellar phase takes a much longer time. At very long equilibrium times (many days) the same equilibrium structure (lamellar phase at the interface) was formed. This observation agrees with presence of a third emulsifier phase at the O/W interface suggested by Friberg et al. (Friberg 1971; Friberg et al. 1969). Westesen has shown the existence of triple layers in lecithin stabilized vegetable oil emulsions using synchrotron X-ray scattering (Westesen and Wehler 1993), but for their system they found that not more than a monolayer is needed for stable emulsions. The addition of β-lactoglobulin has also little effect on the formation and the formed DOPC layer when the DOPC is dispersed in the oil phase.

Bylaite et al. (2001) also studied the stability and droplet size of β-lactoglobulin and lecithin (phosphatidylcholine from sb-PC) stabilized emulsions of caraway essential oil as well as the amount of protein on the emulsion droplets. It should be

noted that sb-PC was dispersed in the oil phase. Some of their data are given in Fig. 5.14, where the amount of β-lactoglobulin adsorbed on the oil aqueous interface is shown versus amount added s-PC. These data show that sb-PC is likely to replace some of the protein at the oil—aqueous interface, although it is unable to completely replace the protein. The maximum reduction in the amount of β-lactoglobulin adsorbed is by a factor of 3 for the caraway oil. These findings are in agreement with other studies, where lecithin was found to be less efficient in displacing milk proteins from the oil/water interface compared to other surfactants (Courthaudon et al. 1991; Dickinson and Iveson 1993). Xue and Zhong (2014) studied the formation of nanoemulsions using mixtures of essential oils, gelatin and lecithin. Nanoemulsions with particle diameters smaller than 100 nm were obtained when mixing both with 0.2 wt% gelatin and 1 wt% lecithin, for which synergetic hydrophobic and electrostatic contributions are thought to promote the emulsification process. These dispersions were stable against aggregation and Ostwald ripening under up to 4 weeks when stored at neutral pH and room temperature.

The displacement of caseinate from the interface of emulsion droplets by monoglycerides, monooleoylglycerol and monostearoylglycerol, dissolved in the oil phase was found to correlate with the adsorption of the monoglycerides at the oil-water interface (Heertje et al. 1990). The amount of monooleoylglycerol increased gradually with concentration and reached a plateau when approaching an oil phase concentration of 1 wt%. Under these conditions all of the caseinate was displaced from the interface. The saturated lipid, monostearoylglycerol, was much more efficient in displacing the protein. Already, at a concentration in the oil phase of between 0.2 and 0.3 wt% the adsorbed amount of monostearoylglycerol increased sharply and reached much higher surface concentrations than monooleoylglycerol. At 0.3 wt% all of the caseinate was removed from the interface.

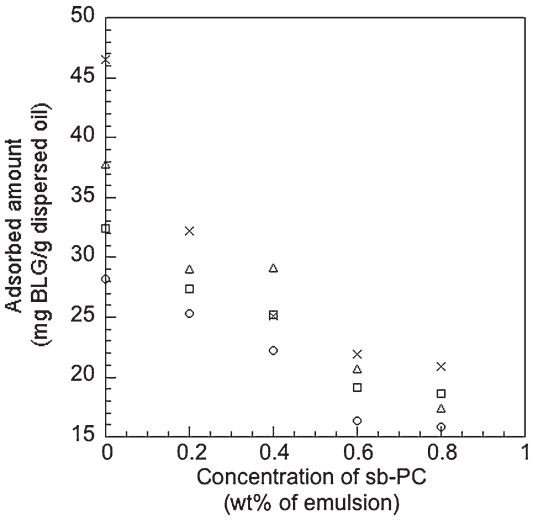

Fig. 5.14 Adsorbed amount of protein at the caraway essential oil – water (Δ, ×) and olive oil –water (◯, □) interfaces in emulsions stabilised by 1 (Δ, ◯) and 2 (×, □) wt.% β-lactoglobulin and variable amount of soybean-PC. Emulsions were prepared from 15 wt.% oil in a 60 mM phosphate buffer of pH 6.7. Data adopted from Bylaite et al. (2001) where also the experimental details are given

Matsumiya et al. (2014) found that coalescence of sodium caseinate oil in water emulsions occur on the addition of dioleate emulsifier, which in turn causes phase separation in oil and aqueous phase under agitation. This was correlated to a partial displacement of the protein stabilizer. Similar detrimental effect on the emulsion stability of combining lipid and protein-based stabilizer was also observed when comparing size distribution of emulsions prepared with milk fat globule membrane (MFGM) extract and buttermilk protein preparations with different protein contents (Miocinovic et al. 2013).

5.4.3.2.1 Protein Interactions with Lipid Vesicles

The mechanisms that determine the stability, size and shape of vesicles are complex and widely discussed, see for instance (Helfrich 1989; Komura 1996; Lasic 1993; Lasic et al. 2001; Mitchell and Ninham 1981). The spherical shape is generally the most stable shape for equal distribution of molecules between the two monolayers constituting the bilayer (Lasic 1993). These spherical vesicles can be large multilamellar vesicles (MLV), and large (LUV) and small (SUV) unilamellar vesicles (Lasic 1993). The bending of the lipid bilayer to form a vesicle imposes a strain on a symmetric bilayer as the inner monolayer has a negative curvature, while the outer has a positive curvature. The magnitude of this curvature energy can be difficult to estimate, but it is thought to be significant enough to in many cases make the vesicles inherently unstable and energy has to be added to form them (Lasic 1993; Komura 1996; Lasic et al. 2001). The result of the tension can be non-spherical vesicles (Seifert et al. 1991). A mixture of phospholipids, which corresponds to the composition in the milk fat globule membrane, gives both spherical vesicles and tubular structures (Waninge et al. 2003). In particular compositions (e.g., 80% DOPE, 12% DOPC and 8% sphingomyelin) that at high lipid content give liquid crystalline phases at the boundary of lamellar to reversed hexagonal phase tend to give microtubular structures at high water content rather than vesicles. A larger proportion of multilamellar vesicles were observed in buffer and divalent salts than in pure water. A small increase in the interlayer spacing of the multilamellar vesicle was observed in the presence of β-lactoglobulin and β-casein.

5.4.3.2.2 Driving Force for the Protein-Vesicle Interaction

The driving mechanism for the interaction of proteins with the lipid bilayer of the vesicles are basically as for the interaction a lipid monolayer at the air-aqueous interface. In parallel to the Quinn and Dawson (1969a, b) study discussed above, Rytömaa et al (1992) found a strong electrostatic contribution when cytochrome c binds to cardiolipin-phosphatidylcholine liposomes. This interaction did not take place if the negatively charged lipid cardiolipin was absent in the membrane. Furthermore, the protein was dissociated from the vesicle in the presence of 2 mM $MgCl_2$ and 80 mM NaCl at pH 7. The apparent affinity of cytochrome c to the

vesicles also increased when the pH was dropped to 4. The interaction was found to be completely reversible for pH changes, that is, if the pH was increased to 7, the protein could be dissociated from the vesicle by adding salt.

Price et al. (2001) studied the adsorption of fibrinogen to neutral liposomes, composed mainly of phosphatidylcholine (PC) and cholesterol and negative liposomes, composed mainly of phosphatidic acid (PA) and cholesterol, as well as to the corresponding liposomes in which a PEG-modified phosphatidylethanolamine had been introduced. They found that negatively charged liposomes adsorbed more fibrinogen than the corresponding neutral liposomes. PEG modification was found to have no effect on neutral liposomes in terms of fibrinogen adsorption. However, PEG modification, which sterically stabilizes the liposomes, markedly reduced the adsorption to the negative liposomes.

Brooksbank et al. (1993) conducted an extensive study on the interaction of β-casein, κ-casein, α_{s1}-casein, and β-lactoglobulin with negatively charged egg yolk phosphatidylglycerol (PG) and zwitterionic egg yolk phosphatidylcholine (PC) vesicle using photon correlation spectroscopy. Their data on the adsorption of β-casein are shown in Fig. 5.15. All of the studied proteins were found to give a thicker layer on the negatively charged vesicles, although they all carried a negative net charge under the conditions used (160 mM sodium chloride at pH 6.2). Brooksbank et al. (1993) suggested that binding to the vesicle surface takes place mainly through hydrophobic interactions and the differences in thickness of the adsorbed layers on the two types of vesicles were explained in terms of the protein charge distribution. For instance the hydrophilic, N-terminal, part of β-casein has a net charge of −12, whereas the remainder of the molecule carries almost no net charge. Thus, on the negatively charged vesicle surface, the molecules adopt a more extended configuration as the N-terminal part is likely to be pushed away from the

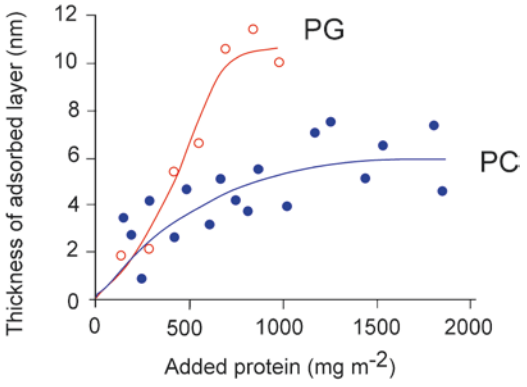

Fig. 5.15 Thickness of adsorbed layer of β-casein on negatively charged egg yolk phosphatidylglycerol (PG) and zwitterionic egg yolk phosphatidylcholine (PC) vesicle as a function added protein expressed as μg of protein per square meter of available liposome surface. The liposomes were dispersed in 160 mM and the pH was about 6.2. The data are taken from a photon correlation spectroscopy study by Brooksbank et al., (1993) where further experimental details are given

surface by means of electrostatic repulsion. This explains the thicker layers on this surface as shown in Fig. 5.15. A similar reasoning can be applied for κ-casein. The apparently very thick adsorbed layer of α_{s1}-casein was explained by bridging flocculation of the vesicles mediated by the protein. The middle section of α_{s1}-casein carries a negative net charge, while the two ends have no net charge. One of the uncharged ends pertrudes into the vesicle bilayer and the middle section is repelled from the vesicle surface, leaving the other uncharged end of the peptide chain free to interact with another vesicle. The charge distribution on β-lactoglobulin is more even and the interpretation of the results was not as straightforward.

As discussed by Kinnunen the introduction of a H_{II} forming double chain lipid (a lipid with packing parameter >1, see Fig. 5.2) in a lamellar membrane can impose a considerable stress on the membrane (Kinnunen 1996). This frustrated membrane is said to be in the L_e state according to the Kinnunen (1996) terminology. Free energy can be gained by allowing some of the lipids in the frustrated membrane to adopt the so-called extended or splayed chain conformation, where one of the acyl chains extends out from the bilayer, while the other chain remains in the membrane. Such an extended chain can also become accommodated within a proper (hydrophobic) cavity of a protein interacting with the membrane (Kinnunen 1996). This is an interesting alternative explanation for the hydrophobic interaction between peripheral proteins and membranes that has been discussed in this review. The splayed chain conformation has also been suggested to be one mechanism for membrane fusion (Kinnunen and Halopainen 2000). This and other implications of the splayed chain confirmation has been discussed by Corkery (2002).

5.4.3.2.3 Influence of the Protein Structure on the Vesicle Interaction

Kim and Kim (1986) studied the interaction between α-lactalbumin and phosphatidylserine/phosphatidylethanolamine vesicles (1:1 molar ratio) versus pH. They found that the interaction, which almost did not exist at neutral pH, increased with decreasing pH. What is interesting to note, is that vesicle fusion, as estimated from increase of the initial rate of Tb fluorescence increase, correlates with the binding of the protein to the vesicles. The binding was suggested to be due to hydrophobic interaction via protein segments penetrating into the lipid bilayer as it was impossible to dissociate it by increasing the pH. This was further confirmed by using proteolytic enzymes, which were found to cut off both ends of the polypeptide chain leaving only the segment that penetrate into the bilayer. This penetrating protein loop was also believed to induce fusion of the vesicles.

The importance of the protein conformation on the interaction with vesicles was also shown in the study of Brown et al. (1983). They found no interaction between native β-lactoglobulin and DPPC vesicles, but β-lactoglobulin, modified by exposing it to a 2:1 mixture of chloroform and methanol, did interact with the vesicles. Moreover, the lipid-protein complex formed had an α-helix content of at least 25–30% larger than for the native protein. The interaction was found to lead to aggregation of the vesicles at pH 7.2, while no aggregates were observed at 3.7. This

was explained by the larger net charge at pH 3.7 (+20) compared to pH 7.2 (−10). These results imply that protein modification, either during processing or by special treatment, can increase the helix content, which in turn can be boosted by lipid interaction. The lipid-protein complexes formed in such a way have been suggested as a way to improve the emulsification processes (Brown 1984; de Wit 1989).

Permeability studies showed that the net lysozyme concentration required to induce leakage from negatively charged lipid vesicles was one order of magnitude higher for lysozyme than for hemoglobin, even though the latter carried a net negative charge, Fig. 5.16 (Ruggeri et al. 2013). This suggests that there are other mechanisms besides the electrostatic attractive interaction arising from the net charge of proteins and lipids that contributed to the enhanced capability of hemoglobin to change the permeability of lipid bilayers. Moreover, lysozyme binding to lipid vesicles led not only to increased permeability but also to protein

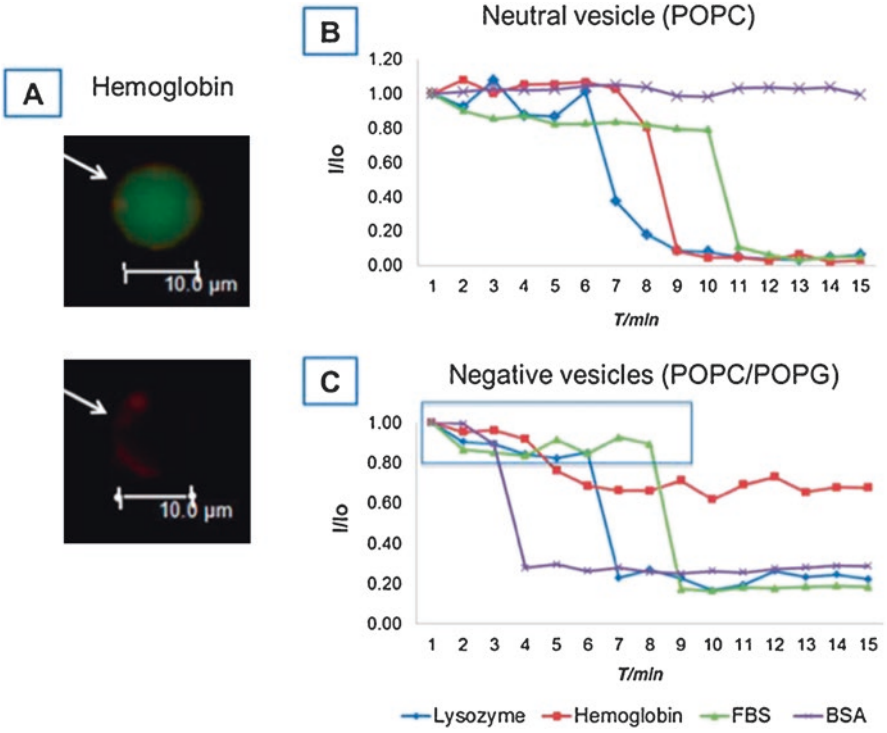

Fig. 5.16 Fluorescence microscopy results showing the effect of soluble proteins on the permeability of giant unilamellar lipid vesicles of a soluble fluorescent dye. A representative image of a single vesicle leakage event is given in (**a**). Representative leakage rate of an individual vesicle expressed as the ratio of real time intensity and initial intensity, I/I_0, versus elapsed time, from when vesicles were exposed to lysozyme (100 µM), hemoglobin (10 µM), Fetal bovine serum (1%) or bovine serum albumin (100 µM). The vesicles carried either neutral charge (**b**) or a net negative charge (**c**). The protein concentrations used are one order of magnitude lower in (**c**). Adopted from Ruggeri et al. (2013). Copyright 2013 by the Royal Society of Chemistry

integration into lipid bilayer and vesicle fusion (Ruggeri et al. 2013; Al Kayal et al. 2012). Indeed it has been suggested that the hydrophobic environment of the lipid bilayer could induce changes in protein conformation, including lysozyme refolding (Nielsen et al. 2010).

5.4.3.2.4 Lateral Phase Separation in Vesicle Bilayers

Raudino and Castelli (1992) reported that the presence of lysozyme could induce lateral phase separation in vesicle bilayers composed of a mixture of phosphatidic acid and phosphatidylcholine. Their differential scanning calorimetry study of the lipid chain melting transition showed good mixing in absence of the protein and the single peak was shifted towards higher temperatures as the phosphatidic acid content increased. In the presence of lysozyme, however, the chain melting transition peak was split into two peaks, indicating a lateral phase separation. In addition they found that temperature of protein unfolding increased with the fraction of phosphatidic acid, suggesting a stabilization of the protein due to the interaction with phosphatidic acid.

It is important to bear in mind that microheterogeneity of the bilayer does not only occur for mixtures of different lipids, but also close to the gel-to-fluid phase transition of the lipid. Hønger et al. (1996) studied the relation between phospholipase A_2 catalyzed hydrolysis of one component phosphatidylcholine vesicles and the microheterogeneity of the lipid bilayer. They varied the microheterogeneity by changing the temperature in the vicinity of the gel-to-fluid phase transition as well as using lipid chain lengths between C14 and C18 and found a strong correlation between the maximal lipase-lipid interaction and the maxima in interfacial area between gel and fluid domains.

5.4.3.3 Liquid-Air Interfaces (foams)

5.4.3.3.1 Emulsifiers with High Aqueous Solubility

Adsorption of emulsifier-protein complexes

Tween 20 and β-lactoglobulin are known to interact in solution to form a 1:1 complex characterized by a K_d = 4.6 μM, which has an increased hydrodynamic radius of 5.7 nm compared to 3.5 nm for β-lactoglobulin alone (Clark et al. 1991b). Detailed measurements of the properties of foam films formed from a constant concentration of 0.2 mg/mL mixed native and fluorescein-labeled β-lactoglobulin as a function of increasing Tween-20 concentration (Clark 1995; Wilde and Clark 1993) have been reported. This study revealed that between molar ratios (R) of Tween 20 to β-lactoglobulin of 0.2–0.9, there was a progressive increase in the thickness of the foam films and a corresponding decrease in the amount of adsorbed protein to an intermediate level of approximately 50% of that which was originally adsorbed. These changes occurred prior to the onset of surface diffusion of the labeled protein

as determined by the FRAP technique at $R = 0.9$ (Coke et al. 1990). One persuasive interpretation of the data is that coadsorption or trapping of the Tween-20/β-lactoglobulin complex in the adsorbed multilayers could account for adsorbed-layer thickening (Clark et al. 1994a), since the complex is known to have an increased hydrodynamic radius (Clark et al. 1991b). However, further studies have showed that the increase in thickness was mainly due to the displacement of the protein by the surfactant. AFM studies showed that surfactant domains were formed which expanded and compressed the protein rich matrix (Mackie et al. 1999) increasing its thickness prior to complete displacement of the protein. This phenomena was observed in all protein surfactant systems despite the absence of specific protein-surfactant interactions (Mackie et al. 2001a; Mackie and Wilde 2005). Comparing non-ionic and ionic surfactants showed that the headgroup nature had specific impacts on the structure of the interfacial film. Non-ionic surfactants generally formed domains in the protein matrix, which expanded as more surfactant was added. However, ionic surfactants (both anionic and cationic) both formed a greater number of smaller domains than non-ionic surfactant (Gunning et al. 2004) and displaced the protein via the minimal expansion of a larger number of domains. Computer simulations also showed similar surface structures when the interaction potentials between the proteins and surfactants were varied (Pugnaloni et al. 2004; Wijmans and Dickinson 1999). This suggested that non-ionic surfactants in general had a net repulsive interaction with adsorbed proteins, probably due to steric repulsion, whereas ionic surfactants had a relatively more attractive interaction with adsorbed proteins. This is probably due to the fact that although the protein has a net charge, they are polyelectrolytes with both negative and positive charges, thus, some parts of the protein will be attracted to an ionic surfactant, irrespective of its charge.

Further evidence supporting direct adsorption of the complex formed between β-lactoglobulin and Tween 20 comes from dynamic surface tension (γ_{dyn}) measurements performed using the overflowing cylinder apparatus (Clark et al. 1993). Inclusion of β-lactoglobulin (0.4 mg/mL) in the initial solutions caused only a small reduction in the measured γ_{dyn} to 7 l mN/m. This remained unaltered in the presence of Tween 20 up to a concentration of 15 μM. Above this concentration a small but significant further reduction in γ_{dyn} was observed. The effect resulted in a small inflection in the γ_{dyn} curve in the region corresponding to 15 to 40 μM Tween 20. At higher Tween-20 concentrations, the curve for the mixed system followed that of Tween 20 alone. The inflection in the γ_{dyn} isotherm observed for the mixed system at concentrations of Tween 20 greater than 10 μM could not be due to adsorption of Tween 20 alone since, under the prevailing conditions, its association with β-lactoglobulin reduced the concentration of free Tween 20. Using Eq. (5.5) it can be shown that the Tween-20/β-lactoglobulin complex is the dominant component in solution in the Tween-20 concentration range of 15 to 35 μM (Clark et al. 1993).

Direct adsorption of complex at the air/water interface also appears to have importance in functional properties of certain lipid-binding proteins from wheat called “puroindolines” (Biswas and Marion 2006; Dubreil et al. 1997; Wilde et al. 1993). These proteins show unusual behavior in the presence of lipids that they

bind, in that their foaming properties are generally unaltered and in some cases enhanced. A systematic study of the influence of interaction with lysophosphatidyl cholines (LPC) of different acyl chain lengths and has produced persuasive evidence of the importance of the complex on foaming activity (Wilde et al. 1993). First, two isoforms of the protein were investigated, puroindoline-*a* and -*b* (the *b* form has also been referred to as "friabilin"). Puroindoline-*b* has a significantly increased K_d for LPC compared to puroindoline-*a* (i.e., 20-fold weaker binding) and the enhancement of foaming properties is correspondingly reduced in the *b* form. Further studies of the binding of LPC to the *a* form revealed that the binding became tighter with increasing acyl chain length, and higher concentrations of the short-chain-length LPC are needed to achieve optimal foam stability enhancement (Husband et al. 1995). Lauryl-LPC showed no interaction with the puroindoline-*a* until the levels present exceeded the critical micelle concentration of 400 μM. This indicates a cooperative binding since it takes place in this concentration range, and any of the suggested structures for the protein/surfactant complexes, e.g., the pearl and necklace structure (Fig. 5.6), could be applicable. It seems increasingly likely that the functional properties of the puroindolines are linked to a role in the transport and spreading of lipid at the air/water interface.

When comparing the data for the interaction between SDS and ovalbumin and the corresponding data for BSA we clearly observe the different mode of interaction (Fig. 5.17b). The gradual decrease in surface tension with increasing surfactant concentration observed for ovalbumin and SDS mixtures can be explained by more efficient packing at the interface as discussed below. In addition, it has been argued that the attractive electrostatic interaction between surfactant and protein might increase the hydrophobicity and hence the surface activity of the protein. The specific binding of SDS to BSA does not affect the surface tension until the concentration corresponding to saturation of the high affinity binding sites is reached, that is 9–10 mole SDS per mole protein (Makino 1979), where a sharp decrease in surface tension is observed. This arises probably from an increase in the free monomer concentration of SDS. The second plateau, indicating constant surfactant monomer concentration, which is observed at increased surfactant concentration, is likely to be connected with saturation of the co-operative binding sites. As surfactant concentration further increases the surface tension isotherms for the two protein surfactant mixtures coincide. The second plateau observed in surface tension isotherms for ovalbumin and HPC mixtures just below cmc of HPC (Fig. 5.17c), can be related to the electrostatic interaction between HPC and globular proteins that has been observed below cmc in bulk solution (Ericsson et al. 1987a). It is noteworthy that the surface tension is slightly lower than for pure HPC, suggesting that the complex is more surface active. Green et al. (2000) used specular neutron reflection and surface tension measurements to study the adsorption of lysozyme and SDS at the air-water interface. Their results show that the lysozyme-SDS complexes are much more surface active than the unbound species as the surface excesses for both lysozyme and SDS increases and surface tension decreases upon addition of SDS (region A). Interestingly, the molar ratio of SDS to lysozyme was found to remain constant at about 7, although the total surface excesses increase with SDS concentration up

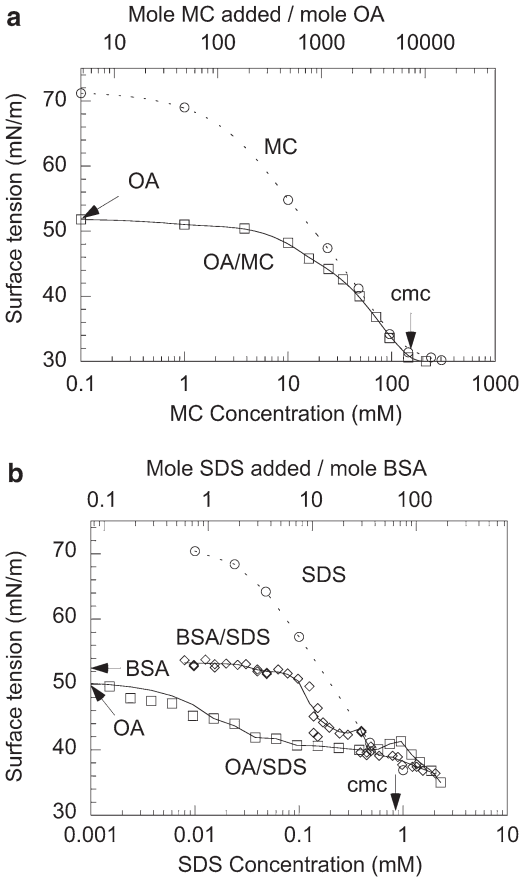

Fig. 5.17 (**a**) Surface tension isotherms of 21 μM ovalbumin (OA) (□) in the presence of the nonionic monocaproin (MC) in water adjusted to pH 5.6, where the surface tension of the pure protein is marked with an arrow on the ordinate. Surface tension of pure MC is also shown (○) and the cmc is marked with an arrow on the abscissa. The surface tension measurements were performed according to the drop-volume method as a function of time. The surface tension value after 2000 s has been used for the isotherms. Further details are given elsewhere (Ericsson and Hegg 1985). (**b**) Surface tension isotherms of 21 μM ovalbumin (OA) (□) and 13 μM bovine serum albumin (BSA) (◇) in the presence of the anionic sodium dodecylsulphate (SDS) in 0.05 M phosphate buffer, pH 5.6. The surface tension of the pure proteins are marked with arrows on the ordinate. Surface tension of pure SDS is also shown (○) and the cmc is marked with an arrow on the abscissa. Other conditions are the same as given under Fig. 5.17a. (**c**) Surface tension isotherms of 21 μM ovalbumin (OA) (□) in the presence of the cationic hexadecylpyridinium chloride (HPC) in water adjusted to pH 4.0. The surface tension of the pure protein is marked with an arrow on the ordinate. Surface tension of pure HPC is also shown (○) and the cmc is marked with an arrow on the abscissa. Other conditions are the same as given in Fig. 5.17a

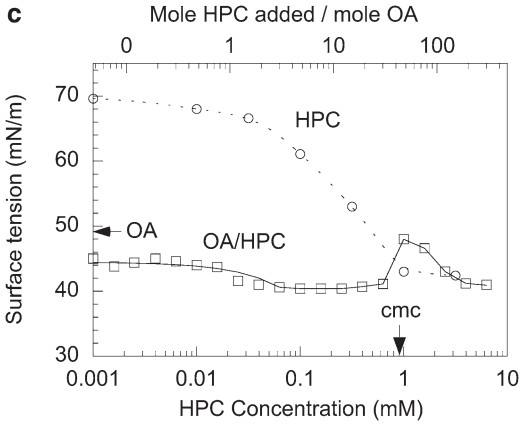

Fig. 5.17 (continued)

to a surfactant concentration of 2.5×10^{-4} M. This indicates that the complex that adsorbed on the interface had a rather well defined stoichiometric composition. Further increase in SDS concentration beyond 2.5×10^{-4} M lead to a sharp decrease in the total surface excess, while the molar ratio of SDS to lysozyme increased. Eventually, as more SDS was added, the mixed protein/surfactant layer was replaced by a pure SDS monolayer. The zwitterionic surfactant LPC was found to enhance the foaming properties of β-lactoglobulin (Sarker et al. 1995). An enhanced adsorption of this complex, and an increase in the elastic properties of the mixed interface were also found, which could be linked with enhanced electrostatic interactions between the adsorbed protein and surfactant (Gunning et al. 2004).

Decreased surface activity of the emulsifier-protein complex

The maxima in the surface tension isotherm, occurring at HPC concentrations between 0.8 and 2.5 mM, probably reflect an increased HPC-ovalbumin interaction in bulk solution (Fig. 5.17b). The formed highly charged complex is less surface active and an increase in surface tension is thus observed. The surface tension maximum has been found to depend on ovalbumin concentration, and is shifted towards higher HPC concentration at increased ovalbumin concentration (corresponds to 30 mole HPC per mole ovalbumin, independent on protein concentration) (Ericsson 1986). The adsorption from mixtures of human serumalbumin (HSA), and non-ionic surfactant, decyl-dimethyl-phosphine-oxide (C_{10}DMPO) at the air-water interface was reported by Miller et al. (2000b). They reported an anomalous surface tension increase for the mixtures at low surfactant concentrations to values higher than for the protein at the same concentration with out the surfactant. Thus it seemed that surfactant protein complex was less surface active. The likely explanation is that the non-ionic surfactant associate with HSA via hydrophobic interaction and thus makes the protein more hydrophilic and hence less surface active. Miller et al. (2000b) also observed that the concentration range,

where the coverage of protein and surfactant are comparable in the mixed surface layer was quite narrow.

The precipitation of protein in the bulk solution due to neutralisation by added surfactant can also cause a decrease in surface concentration due to loss of surface active material. Garcia Dominguez et al. (1981) showed that the surface tension reduction of lysozyme and insulin at pH 3.5 (i.e. below pI) decreased when an anionic surfactant (SDS) was added, due to precipitation of the protein.

The lateral electrostatic interactions can control the layer composition

A synergistic effect on surface tension is seen for mixtures of proteins with both the anionic and cationic surfactant (Fig. 5.17b, c). For ovalbumin and SDS mixtures (Fig. 5.7b), a gradual decrease of the surface tension with increasing surfactant concentration is observed. This might be assigned to the more efficient packing in the formed mixed surfactant/protein layer compared to the one formed by the individual components at this concentration (Ericsson and Hegg 1985). Even at the lowest concentration of cationic surfactant (0.05 mole HPC per mole ovalbumin), where the pure surfactant has the same surface tension as water, a decrease in surface tension for the protein surfactant mixture, compared to pure ovalbumin, is observed (Fig. 5.17c). It is unlikely that any bulk interaction will affect the interfacial behaviour at this low HPC to ovalbumin ratio. Therefore the lowering in the surface tension probably arises from molecular interactions in the adsorbed surface film, giving a more condensed surface layer. Buckingham et al. (1978) found strong synergistic lowering of the surface tension of a mixed solution of SDS and poly-L-lysine at conditions at which no precipitation, micelle or complex formation take place in the bulk solution. Similar behaviour was observed in mixtures of low molecular weight surfactants of opposite charges (Lucassen-Reynders et al. 1981). This effect has been assigned to the reduction of electrostatic repulsion within the layer.

5.4.3.4 Emulsifiers with Low Aqueous Solubility

The large number of studies using lipid monolayers at the air/aqueous interface and spread or adsorbed proteins have given us the basic knowledge of the interaction between proteins and polar lipids with low aqueous solubility.

5.4.3.4.1 Monolayer Stability

One might expect that monolayer made up of lipids with very low aqueous solubility would be stable. However, this is far from general. Metastablility of monolayers, can be caused by processes such as rearrangement within the layer, dissolution into the sub-phase and transformation to a three dimensional phase, which can occur at pressures above the equilibrium spreading pressure (Vollhardt 1993; Vollhardt et al. 1996). Furthermore, the stability of the monolayers can be affected by the spreading solvent and the techniques used for spreading the lipid (Carlsson et al. 1995; Gericke

et al. 1993). The stability of the monolayer can also be considerably changed by the ion composition of the aqueous sub-phase. For instance the stability of an arachidic (n-eicosanoic, C20:0) acid monolayer was found to increase in the order $H^+ < Li^+ < Na^+ < Ca^{2+} < Mg^{2+}$ (Vollhardt 1993).

There are several examples of proteins that are thought to have the role to stabilize a lipid mono- or bilayer. One such example is the milk fat globule membrane that has been suggested to consist of the monolayer of polar lipids, which cover s fat globule surface, and an outer lipid based bilayer (Danthine et al. 2000; Mather 2000). The milk fat globule membrane is expected to be inhomogeneous with significant amount of proteins in the membrane. An aqueous layer containing different proteins, like xanthine oxidase, is present between the monolayer and bilayer. One of the roles that have been assigned to xantinoxidase is to stabilize the milk fat globule membrane (Mather 2000). Interestingly, Kristensen et al., (1996) found that the presence of a xanthine oxidase can increase the stability of a monolayer composed of sphingomyelin from the milk fat globule membrane. They investigated the interaction between one of the major proteins, xanthine oxidase, and the major lipids, sphingomyelin and phosphatidylcholine, in the milk fat globule membrane at the air/aqueous interface by using the monolayer technique. Both lipids have a similar phosphorylcholine headgroup, which is zwitterionic in the neutral pH range, although the belt regions linking the phosphorylcholine group with the acyl chains are different. The Π-A isotherms of sphingomyelin and phosphatidylcholine are shown in Fig. 5.18a, b, respectively. The isotherms for sphingomyelin monolayers spread on pure buffer and a xanthine oxidase solution are shown. The

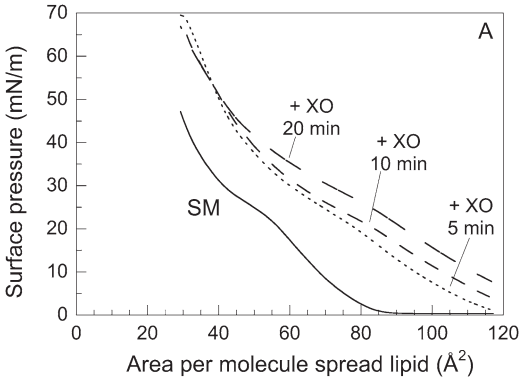

Fig. 5.18 Dynamic surface pressure (Π) as a function of the molecular area of the spread amount lipid for compression of (**a**) sphingomyelin and (**b**) distearoylphosphatidylcholine (DSPC) monolayers on a phosphate buffered subphase (40 mM phosphate containing 0.1 M sodium chloride, pH = 7.4) with or without xanthine oxidase (5 mg/ml). The isotherms recorded for the lipid spread on pure buffer ( ) and at 5 (- - - - -), 10 (— — — —), 20 (— - — -) min elapsed between spreading on xanthine oxidase solution and compression. The lipid (25 μg) was spread from a chloroform/methanol (2:1, v/v) solution on a maximum area of 50 × 450 mm^2 and a compression speed of 12.5 mm/min was used. Data adopted from Kristensen et al. (1996), where also the experimental details are given

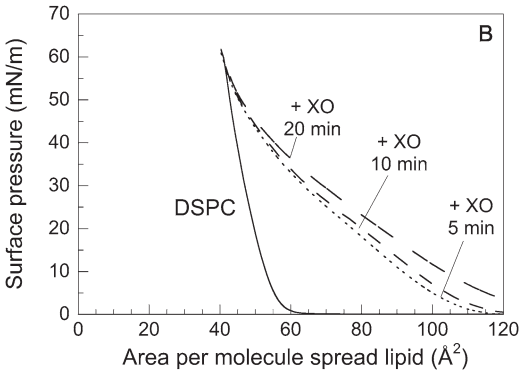

Fig. 5.18 (continued)

slope of isotherm and the area of the compressed monolayer for pure sphingomyelin (Fig. 5.18a) are smaller than expected for these types of lipids. In addition, the large hysteresis and the dependence on the compression speed, not observed for distearoylphosphatidylcholine, confirms that the sphingomyelin monolayer is metastable. The difference in stability of monolayers formed by two different lipids can probably be related to the different conformation of choline groups in the two types of lipids, where intra molecular hydrogen bonding is possible between the phosphate group and the amide and hydroxyl groups in the belt region of sphingomyelin (Siminovitch and Jeffrey 1981). An increase in Π at maximum compression of the sphingomyelin monolayer, which reflects an increase in the monolayer stability, was observed in the presence of sphingomyelin. Furthermore, the area per sphingomyelin molecule increases in the presence of xanthine oxidase even at high Π-values. This is in contrast to the results from the parallel study of the phosphatidylcholine monolayers with and without xanthin oxidase, where the interacting protein could be completely squeezed out from the lipid monolayer at high enough surface pressures without affecting the collapse pressure. This indicates that interaction between xanthine oxidase and sphingomyelin is much stronger than that between the protein and phosphatidylcholine.

5.4.3.4.2 Structure of the Interfacial Film

Even from the study of the penetration of protein versus surface pressure it is also possible to get some hints about the structure of the mixed layer. Cornell et al. (Cornell 1982; Cornell and Patterson 1989; Cornell et al. 1990) observed penetration of β-lactoglobulin, α-lactalbumin or BSA into mixed monolayers of POPC and POPG at such high surface pressure that it is unlikely that the proteins could penetrate into a protein layer. Thus, they concluded that the formation of pure protein patches is unlikely and that portions of the protein may be intercalated into the lipid

monolayer. Bos and Nylander (1995) made similar observation for the interaction between β-lactoglobulin and DSPC and DSPA monolayers.

Fluorescence microscopy and Brewster angle microscopy (BAM) can be used to in situ image the structure of the film at the air/aqueous interface, although the lateral resolution is limited by the resolution of the optical microscope. Fluorescence microscopy together with surface film balance technique was used by Heckl et al. (1987) to study the structure of mixed phospholipid -cytochrome c and b films. They found that proteins mainly were located in the fluid membrane phase, which coexisted with solid lipid domains without protein. The penetration into the lipid monolayer was reduced with increasing pressure. Cytochrome c (positively charged) was found to interact with dimyristoylphosphatidic acid (DMPA) monolayers but not with dipalmitoylphosphatidylcholine (DPPC) layers, showing the electrostatic nature of the interaction. Schönhoff et al (1992) concluded from their study of the incorporation of membrane proteins into DPPA/DOPA monolayers that incorporation mainly takes place in the fluid phases of the matrix. Zhao et al. (2000) used BAM to image the kinetics of β-lactoglobulin penetration into DPPC monolayers at the air-aqueous interface from a 500 nM solution in 10 mM phosphate buffer, pH 7. For instance at an initial surface pressure of 7.8 mN/m, it took 0.17 min until domains, with similar morphology as those appearing during the compression of a pure DPPC monolayer, appeared. These domains were found to consist only of the lipid as confirmed by grazing incidence X-ray diffraction and β-lactoglobulin penetration was found to occur without any specific interaction with DPPC. β-Lactoglobulin was not able to penetrate into a condensed DPPC monolayer, that is, above surface pressure of about 20 mN/m.

The lateral organization in mixed protein-lipid films at air-aqueous interface can be studied by spectroscopic techniques and high resolution imaging techniques such as electron microscopy and atomic force microscopy (AFM) after transferring the films to a solid support. Using electron microscopy Cornell and Carroll found that only lipids with the chains in liquid state, e-PA, dioleoylphosphatidylcholine and dioleoylphosphatidylethanolamine, formed homogenous films with β-lactoglobulin, whereas DPPA and DSPC formed heterogeneous layers (Cornell and Caroll 1985). AFM as powerful technique to study the lateral organization in mixed films of proteins and soluble surfactant s have already been demonstrated with the development of the “orogenic” displacement model (Mackie et al. 1999, 2001b, b). Diederich et al. studied the interaction between bacterial surface layer proteins (S-layer proteins) and phosphatidylethanolamine (DMPE and DPPE) monolayers using dual label fluorescence microscopy, FTIR spectroscopy, and electron microscopy (Diederich et al. 1996). When the monolayer is in the two-phase region, with one isotropic and one anisotropic fluid phase, the S-layer protein adsorbed preferentially to the isotropic phase. However, 2D crystallization could be nucleated in the boundaries between the two phases, but preceded mainly underneath the anisotropic phase. The FTIR-measurements clearly indicate that the protein crystallization leads to an increased order of the lipid acyl chains.

5.5 Applications

Not only the composition of the interfacial layer, but also the mechanical properties, e.g. the dilational viscosity, of the layer is important for the stability of emulsions and foams (MacRitchie 1990; Prins and Bergink-Martens 1992; Dickinson 1999; Bos and van Vliet 2001). In particular, both surface and bulk rheology as well as the disjoining pressure of the thin lamellae determine the stability of foams (Dickinson and Stainsby 1982; Bos and van Vliet 2001). Hence, in technical applications thickeners are often added. The mechanical properties of interfacial films can to a large extent be controlled by the intermolecular interactions. Protein stabilisation of a foam is mainly due to protein-protein interaction and the destabilisation is thought of as a disruption of these interactions according to the Gibbs-Marangoni effect discussed above in the beginning of Sect. 5.4.

5.5.1 Role of Protein—Emulsifier Interactions in Real Food (and Pharmaceutical) Systems

How the basic principles defined in Sect. 5.4 come into play in some typical applications

Sarker et al. (1995) discussed the effect of the surfactant properties on the stability of interfacial films in foams. The addition of small amount of lysophosphatidylcholine (LPC) was found to increase the foam stability of β-lactoglobulin foams (Sarker et al. 1995). A further increase of the surfactant concentration led to a decrease of the foam stability. The surface tension versus molar ratio of LPC and β-lactoglobulin show an inflection point close to unity molar ratio, corresponding to the binding of the surfactant to the protein. No increase of foam stability was, however, observed for mixtures of Tween 20 and β-lactoglobulin, instead the stability decreased with increasing surfactant concentration (Coke et al. 1990). The same observations was made for the stability of an oil-in-water emulsion, where it was found that small amount of Tween 20 increased the rate of shear induced coalescence of β-lactoglobulin stabilised emulsion droplets (Chen et al. 1993). The marked reduction in surface shear viscosity even at low surfactant to protein ratios confirmed that loosening of the protein layer occurred. The effects of LPC on interfacial rheology showed that at low surfactant to protein ratios, an enhancement in the surface elasticity was found (Gunning et al. 2004), which could explain the observed increase in foam stability. The protein-surfactant complex is thought of being less surface active and a further increase of the surfactant concentration will lead to replacement of protein and protein surfactant complexes with surfactant at the interface (Coke et al. 1990; Krägel et al. 1995). The mobility of the protein in a protein stabilised thin liquid film, as measured with the fluorescence recovery after photobleaching technique (FRAP), increases at lower surfactant to protein ratio for Tween 20 than for LPC (Fig. 5.19). This was attributed to the stronger binding of Tween 20,

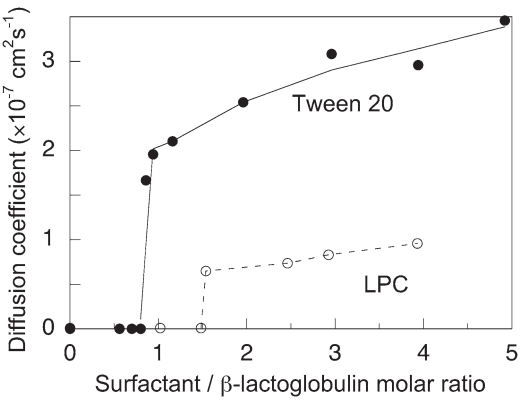

Fig. 5.19 The effect of surfactant addition on the lateral diffusion in the adsorbed mixed layer of surfactant and β-lactoglobulin, measured with the fluorescence recovery after photobleaching, FRAP, technique. The diffusion coefficients of the fluorescent probe 5-N-(octadecanoyl) aminofluourescein and fluourescein isothiocyanate isomer 1 labelled β-lactoglobulin measured in the presence of L-α-lysophosphatidylcholine (○) and Tween 20 (●), respectively, are shown as a function of the molar ration between surfactant and β-lactoglobulin. The data are adopted from the work of Sarker et al. (1995) and Coke et al. (1990), respectively, in which the experimental details also are given

compared with LPC, to β-lactoglobulin (Sarker et al. 1995) and will also explain why the foam becomes unstable at lower surfactant concentration when Tween 20 is used. The foaming properties of puroindoline from wheat was also found to be improved by the addition of LPC (Wilde et al. 1993). Once the surfactant concentration becomes large enough, the protein-protein interactions within the surface film will be prevented, the mobility increased and thus the foam stability decreased according. These destabilization phenomena are utilized in aerated emulsion based foods such as ice cream and whipping cream, where air, water, and fat surfaces exist and interact. During aeration, proteins initially stabilize the air-water interface, but are gradually destabilized to promote partial coalescence between fat droplets and air bubbles to form a network of partially coalesced fat that stabilizes the foam (Goff 2002). This phenomena is also being investigated to understand how emulsions can be structured during the digestion process to influence the digestibility of food, which could in turn impact on appetite and overall food intake to tackle obesity (Day et al. 2014).

The lipid binding activity of puroindolines can be exploited to counteract the foam damaging effects of lipids. Lipid binding proteins can sequester lipids and prevent their adsorption and subsequent destabilizing of protein foams (Clark et al. 1994b). These proteins are common in cereals and may play a role in foam stability in baked products and beer.

Ionic surfactant can also induce flocculation of protein-stabilised emulsions and this is depending on the nature of the protein-lipid interaction as discussed by Chen and Dickinson (1995a, b, c). An anionic surfactant, sodium lauryl ether sulphate

(SLES), at sufficient concentration has been found to flocculate gelatine stabilised oil-in-water emulsion (Chen and Dickinson 1995a). A further increase in surfactant concentration was found to lead to a restabilization of the flocculated emulsion. In bulk solution the anionic surfactant will, at high enough concentrations, cause precipitation of the positively charged gelatine. At a further increased surfactant concentration, the precipitate was redispersed. Gelatine was initially displaced by SLES from the interface (Chen and Dickinson 1995a), but an increase of the surfactant concentration leads to an increase of gelatine concentration at the interface and the surface charge became partly neutralised (Chen and Dickinson 1995b) causing flocculation. A further increase of the surfactant concentration leads to a decrease of the gelatine surface concentration (Chen and Dickinson 1995c) and a restabilisation of the emulsion (Chen and Dickinson 1995a). It was also observed that the addition of SLES to a β-lactoglobulin stabilised emulsion did not cause any flocculation although some kind of complex was formed in bulk solution. It should be born in mind that β-lactoglobulin was negatively charged under the used experimental conditions. This confirms the electrostatic nature of the observed SLES induced flocculation of the emulsions stabilised by the positively charged gelatine. Flocculation of β-lactoglobulin stabilised emulsions was, however, observed in the presence of gelatine and SLES. Since it only occurred above the cmc of the surfactant it was suggested to depend on cross-linking of the emulsion droplets by surfactant micelles (Chen and Dickinson 1995a).

Bylaite et al. (2001) found that emulsions with triglyceride oil generally proved to be more stable compared to those made with caraway essential oil as the dispersed phase. However, the stability of the emulsions could be improved considerably by adding sb-PC. An increase in the protein concentration also promoted emulsion stability. Fang and Dalgeish (1996) arrived at a somewhat different conclusion for casein stabilized emulsions. They found that the presence of DOPC destabilized casein stabilized emulsions of soybean oil in a 20 mM imidazole/HCl at pH 7.0. This seemed to be independent on whether DOPC was present during emulsification or if it was added to the emulsion as dispersed aggregates. At high concentration of casein, the emulsions were stable, and the decrease in surface load was a direct indication of the removal of casein from the interface by the presence of DOPC. The higher the DOPC concentration, the greater was the effect on emulsion stability and surface load. DPPC and egg PC either enhanced or did not affect the stability of the emulsion.

Waninge et al. (2005) studied the interaction between β-lactoglobulin and β-casein and milk membrane lipids at the oil-aqueous interface in emulsions. They found that the membrane lipid emulsified emulsions were dominated by the membrane lipids even after equilibrium with protein solutions. Protein displacement was not observed for β-lactoglobulin with time in contrast the displacement effects observed for the emulsions with β-casein, when both membrane lipids and β-casein were included during the emulsification. Based on results from three different types of emulsions, formed with different mixing order of the emulsifiers, they arrived on different alternative models that are described in Table 5.2. The eight different models can be divided into two main groups, where models I-III are independent of

Table 5.2 Models describing the oil-water surface with membrane lipids and β-casein/β-lactoglobulin

Model I: A mixed monolayer including both protein and membrane lipids.	
Model II: A mixed monolayer with strong specific interactions between the protein and the lipid.	
Model III: A laterally separated monolayer.	
Model IV: A protein layer adsorbed on top of the lipid layer.	
Model V: A lipid monolayer formed at a protein layer.	

Model VI: A lipid bilayer formed on top of a protein layer.	
Modell VII Vesicular aggregates attached at the interfacial protein layer.	
Model VIII Vesicular aggregates immersed into the interfacial protein layer.	

Adopted from Waninge et al. (2005)

mixing order describing an equilibrium structure and models IV are dependent on mixing order describing and therefore represent nonequilibrium structures. Based on the results obtained from the serum depletion method Waninge et al. could estimate the surface composition at the oil-water interface.

Model *I–III* assumes a mixed monolayer, which is expected to correspond to a coverage of 2–2.5 mg/m^2. Significantly higher total adsorbed amount was observed when the emulsion was prepared in presence of both protein and lipid. Furthermore strong effect of the mixing order was observed, which exclude models I–III. A structure corresponding to model *IV* is the only possible explanation for adsorption of protein to a membrane lipid emulsified emulsion but it may also occur when both components are emulsified together. However, the low protein adsorption (up to about 0.3 mg/m^2) observed when adding protein to the emulsion prepared in the presence of the lipid indicate a structure corresponding to model *IV*.

For both the β-casein and β-lactoglobulin emulsified emulsions significant amount of membrane lipids were observed (around 1.4 mg/m^2) after adding the vesicles. Model *V, VI, VII* and *VIII* may describe the observed association between these emulsions and added membrane lipid vesicles. However, the pronounced hydrophilicity of the milk proteins makes a hydrophobic adhesion of a complete monolayer on top of the protein layer unlikely (Model *V*). The fact that lipid adsorption is observed without a corresponding desorption of protein excludes model *VIII*. Cryo-TEM images showed a few structures in agreement with model *VII*, but the frequency was too low to fully explain the association observed. Model *VI* can be a result of a transition from model *VII*, thus, a combination of model *VI* and *VII* seems to be the most likely structure in the system.

The observed gradual displacement of β-casein when emulsified together with the membrane lipids suggests the presence of the membrane lipid directly at the oil-water interface. Since the total adsorbed amount is well above monolayer coverage, model *VIII* appears more likely than model *III*. However, it can be assumed that the system gradually transforms from a structure of type *VIII* over to the more simple structure of type *III*.

The stable adsorbed layer when the emulsion is emulsified with both β-lactoglobulin and membrane lipids present suggests one of the structures *VI*, *VII* or *VIII*. However, model *VI* and *VII* seem more unlikely as the protein surface load is lower than the surface load of the pure protein emulsified emulsion. Notable is the absence of clear signs protein displacement. A possible explanation is that the protein layer is strongly crosslinked, as previously observed by (Chen and Dickinson 1993; Chen et al. 1993; Mackie et al. 1999).

Several examples of how the properties of the oil phase composition can affect the structure of the adsorbed layer of protein on the emulsion droplet, and hence the stability of the emulsion, have been studied. For instance, the work of (Leaver and Dagleish 1992) on the structure of adsorbed layers of β-casein on emulsion droplets, where it was found that the cleavage of the protein on the oil-droplet surface by trypsin gave different products depending on whether a triglyceride oil or tetradecane, was used. This demonstrates that the structure of the adsorbed layer depends on the composition of the oil.

5.5.2 *Enzyme Activity and Protein/Emulsifier Application*

What effect does the enzyme action have on the self-assembled emulsifier structure? How can lipase activity be affected by the presence of other proteins?

The action of lipolytic enzymes is of importance in a number of food applications or related areas, ranging from their use in detergents, as tools in modifying lipids to the breakdown of acylglycerides both as unwanted side effects and the naturally occurring process in the human intestine (Schmid and Verger 1998). It is well known that lipases work mainly at an interface and therefore one often speaks of "interfacial activation" in connection with lipase activity (Verger 1997; Panaiotov and Verger 2000). They are therefore an important example of lipid/protein interactions at interfaces. Lipases do play an important role in gastrointestinal tract for digestion of fat (Patton and Carey 1979), but they also have come to an increase use in industrial processes, including detergency and food processing (Svendsen 2000).

Lipase act at such a low concentration that their presence as protein does not significantly affect the global lipid self-assembly structure. It is rather their catalytic activity that has an impact on the lipid self-assembly structure. It is also important to remember the action of lipases only decreases the time taken to reach the equilibrium and does not affect the equilibrium composition as such. Thus, the changes in structure in composition would have occurred even without the lipase if given enough time.

Here we will highlight some aspects in relation to the mechanisms of protein/lipid interactions. There are several types of lipases that act on phospholipids and triglycerides, but we will mainly discuss lipases catalyzing the hydrolysis of the ester bonds of triacylglycerols. A number of lipase activity studies have concerned the interfacial reactions of these enzymes using monolayers and have provided some leads on how to control lipase activity by modulating the lipid composition (Golding and Wooster 2010; Reis et al. 2009). The corresponding studies on bulk dispersion can be made by conjugating the lipase (*Thermomyces lanuginosa* lipase (TLL)) to gold nanoparticles in order to visualize the enzyme location and the enzymatic digestion of lipid aggregates by means of Cryo-TEM (Brennan et al. 2010). The enzymatic activity is determined by the concentration of lipolytic enzymes associated with the lipid film and can be inhibited by various proteins (Gargouri et al. 1984b, b). Experiments carried out with mixed protein/dicaprin films transferred over pure buffer yielded evidence that inhibition of hydrolysis was caused by proteins bound to the dicaprin film rather than by a direct interaction between protein and lipase in the bulk phase (Gargouri et al. 1985, 1986). Furthermore, since some lipases were inhibited by adsorption of proteins at the lipid layer, whereas other lipases were still able to hydrolyze a mixed protein/phospholipid layer, indicating that the inhibition of some lipases cannot be attributed merely to steric effects hindering accessibility to dicaprin molecules within the film. Surface concentration measurements of inhibitory proteins showed that only 5–9% of the area of a mixed lipid/protein film was covered by inhibitory proteins, implying that long-range electrostatic forces are likely to be involved in the inhibition as well as

parameters such as surface viscosity and surface potential. However, similar inhibitory effects caused by melittin (pI>10) and β-lactoglobulin A (pI = 5.2) at pH 8.0 strongly suggest that the nature of the inhibition is not an electrostatic phenomenon, but might be assigned to the effect on the properties of the hydrocarbon moiety of the lipid (Gargouri et al. 1987, 1989; Piéroni et al. 1990). The correlation between inhibition of lipase activity and the ability of the inhibitory protein to penetrate into the phospholipid monolayer support this suggestion.

In a simple experiment Wallin and Arnebrant (1994) demonstrated that a cubic phase was much faster decomposed by the action of lipase from *Thermomyces* (former *Humicola*) *lanuginosa* than the reference sample consisting of triolein and aqueous phase. This was attributed to the much larger interfacial area in the cubic phase. In an in vitro study of lipolysis of triglycerides in a intestinal-like environment, Patton and Carey (1979) observed, apart from the initially occurring crystalline phase, a viscous isotropic phase composed of monoglycerides and fatty acids, which is identical to the one formed in monoglyceride systems. In excess of bile salts, the lipolysis products are rapidly solubilised in mixed micelles. However, the bile acid amounts in vivo are not sufficient to solubilise all lipids after a meal rich in fats, which implies that the liquid crystalline phases exist in vivo (Lindström et al. 1981). Lipase and water must be free to diffuse through the phases formed by the lipolysis products, surrounding the diminishing fat droplet. Thus, the bicontinuity as well as the incorporation properties of the cubic monoglyceride phases are thought to be important features for the lipolysis process (Patton et al. 1985). Since then several studies on the liquid crystalline phase and colloidal transformations during the digestion of lipid assemblies, emulsions of acylglycerides or liquid crystalline nanoparticles containing polar lipids have been presented (Barauskas and Nylander 2008; Borné et al. 2002b; Fong et al. 2014; Salentinig et al. 2010, 2011, 2013; Wadsater et al. 2014; Warren et al. 2011; Barauskas et al. 2016). These studies have shown that apart from the lipid composition and the type of lipolytic enzyme, the solution conditions such as bile salt concentration, pH (i.e., the degree of protonation of the fatty acids), and buffer conditions are important both for the kinetics of the lipolysis and the formed nanostructure. Complex protein-surfactant-lipid interactions at the interface are key for lipase activity during digestion. In the stomach, gastric lipase activity is gradually inhibited by a surface layer of lipolytic product at the interface. This is solubilized by bile salts in the duodenum, but the bile salts also inhibit pancreatic lipase. Thus the interaction between pancreatic lipase, colipase and bile salts produces a highly surface active complex which can remain at the interface, even in the presence of bile salts and continue to hydrolyse the acylglycerols (Reis et al. 2009; Wilde and Chu 2011). Borné et al. has in a series of studies investigated the affect of lipase action on liquid crystalline phase as well as other self-assemble structures such as vesicles and cubosomes (Borné et al. 2002a, b; Caboi et al. 2002). Some of their findings are summarized in Fig. 5.20, which shows a schematic representation of the change in structure of the different liquid crystalline phases as a function of time after adding *Thermomyces lanuginosa* lipase. The observed changes in self-assembled structures could be predicted from either the monoolein—oleic acid—aqueous ternary phase diagram, where the lipolysis give

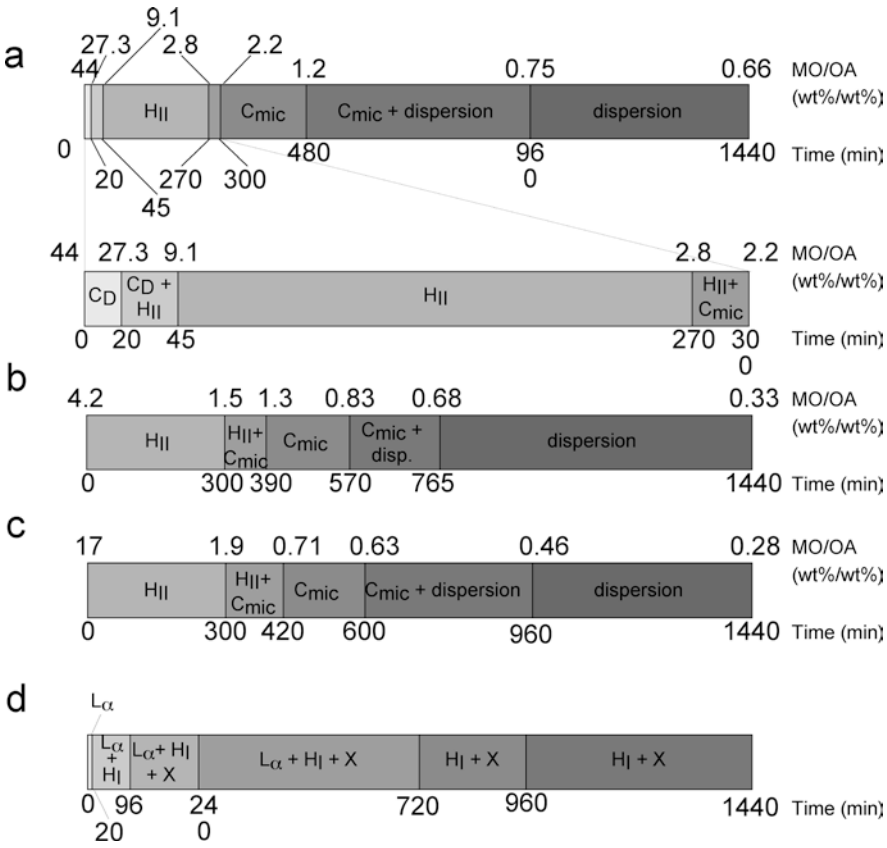

Fig. 5.20 Schematic representation of the change in structure during lipolysis of monoolein (MO) (or diolein DO) in different lc phases: (**a**) C_D phase (63 wt% MO, 37 wt% $2H_2O$), (**b**) Oleic acid (OA)-H_{II} phase (65.4 wt% MO, 15.6 wt% OA, 19 wt% $2H_2O$), (**c**) DO-H_{II} phase (68 wt% MO, 18 wt% DO, 14 wt% $2H_2O$) and (**d**) L_α-phase (10 wt% MO, 5 wt% Sodium oleate (NaO), 85 wt% $2H_2O$). The main liquid crystalline phases as determined by small angle X-ray diffraction (SAXD), are indicated in the figure as diamond type of bicontinuous cubic phase, space group Pn3m, (C_D), reversed hexagonal phase (H_{II}), normal hexagonal phase (H_I), lamellar phase (L_α) and micellar cubic phase, space group, Fd3m (C_{mic}). These may exist in excess of water or in the presence of minor amounts of other phases. Some of the observed reflections in the diffractograms, obtained by SAXD, could not be unambiguously assigned to a structure. This unidentified structure is denoted X. Figure adopted from Borné et al. (2002a) where details are given

rise to a transition of cubic → reversed hexagonal → micellar cubic → reversed micellar phase + dispersion or monoolein—sodium oleate—aqueous ternary phase diagram, where the corresponding sequence is lamellar → normal hexagonal. These difference in reaction sequences could be rationalized in terms of differences in degree of protonation of the fatty (Borné et al. 2002a). The initially lamellar phase had a high pH (about 10), that is a low degree of protonation and thus the degradation as expected follows the monoolein—sodium oleate—aqueous ternary phase

diagram. The initially cubic and hexagonal phase had low pH (4–7), that is a high degree of protonation and thus the degradation as expected follows monoolein – oleic acid-aqueous ternary phase diagram. Adding *Thermomyces lanuginosa* lipase to aqueous dispersions of cubic phases (cubosomes) and lamellar dispersions (vesicles) at high water content and gave the corresponding morphological changes as for the liquid crystalline phases (Borné et al. 2002b). The phase diagrams of the relevant systems can thus be used as maps to navigate through the changes in the self-assembly structure of the substrate and the product. Salentinig et al. (2010) also investigated the impact of the lipolysis products, e. g. oleic acid, on the structure of monoolein cubic phase, by using dispersions stabilized by the Pluronic F127. Their SAXS data show that the system undergoes structural transitions from a dispersion of bicontinuous cubic phases (cubosomes) through dispersions of reversed hexagonal phases (hexosomes) and micellar cubic phases (Fd3m symmetry) to emulsified microemulsions with increasing oleic acid concentration as previously reported (Barauskas and Nylander 2008; Borné et al. 2002b). Even when starting with a β-lactoglobulin and β-casein stabilized triglyceride emulsions, in vitro lipolysis by pancreatic lipase caused a transition sequence from an oil emulsion to a microemulsion, micellar cubic, inverse hexagonal, and finally bicontinuous cubic liquid crystalline droplets (Salentinig et al. 2011). They also observed strong effects on the lipolysis reaction of solution properties such as bile-juice concentration and pH as well as of hydrophobic additives. Salentinig et al. (2013) demonstrated by time-resolved synchrotron SAXD and cryo-TEM that highly ordered nanostructures can be formed during the digestion of milk fat globules catalyzed by lipolytic enzymes. Wadsäter et al. (2014) showed how the structure of the glyceroldioleate/Soy-PC LCNPs, evolves during the exposure to a triacylglycerol lipase (TGL) under near-physiological temperature and pH conditions. TGL catalyzes the degradation of glycerodioleate to monoglycerides, glycerol, and free fatty acids. During the degradation, the internal liquid crystalline structure of the nanoparticles changes continuously from the reversed *Fd3m* structure to structures with less negative curvature (hexagonal, bicontinuous cubic, and sponge phases) and finally results in the formation of multilamellar liposomes. In a follow up study, the results from the action of two types of lipolytic enzymes where compared in terms of the structural evolution (Wadsäter et al. 2018). Since phospholipase A2 (PLA2) catalysis degradation of the phospholipid component, SPC, and porcine pancreatic triacylglycerol lipase (TGL) the hydrolysis of the diglyceride, GDO. Evolution of the structure was found to be very different. PLA2 hydrolyses the lamellar forming component, SPC, and the end result is therefore a reversed micellar lipid phase. However TGL that hydrolysis the reverse phase forming compound, GDO, promotes a lamellar phase. Borné et al. (2002a) found similar specific activity of *Thermomyces lanuginosa* lipase on the cubic phase as on the reversed hexagonal monoolein based liquid crystalline phases, which was somewhat unexpected.

5.5.3 *New Products and Concepts of Using Protein/Emulsifier Interactions*

Food nanotechnology and delivery of functionality

The monoolein-aqueous system is thoroughly studied example of nanostructured system, where two types of cubic phases have been observed on the water-rich side of the lamellar phase (Larsson 1983; Hyde et al. 1984; Landh 1994; Briggs et al. 1996; Qui and Caffrey 2000). Here we will highlight some of the main features that are of importance for the functionality and application of lipid-based liquid crystalline cubic phases. First it is the bicontinuity of the cubic phase. This is illustrated in Fig. 5.21a, b, where the mobility of glucose solubilised in the aqueous channels and vitamin K, solubilised in the lipid bilayer, respectively is illustrated. Figure 5.21a shows the concentration profiles of glucose in the cubic monoolein-aqueous phase equilibrated against water as determined by holographic laser interferometry (Mattisson et al. 1996). These profiles could be fitted to Ficks second law, which gave a diffusion coefficient 4 times lower than the value in aqueous solution. The mobility of the molecules in the aqueous channels of the cubic phase

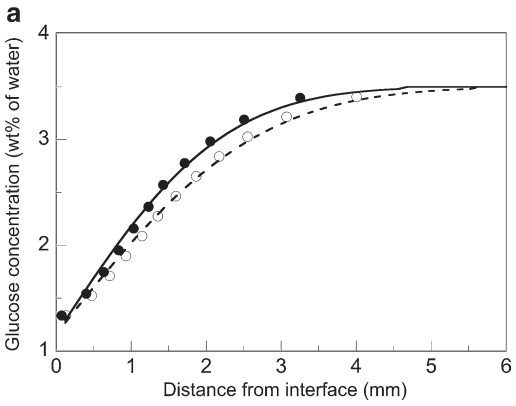

Fig. 5.21 (**a**) Glucose concentration profiles in a monoolein- aqueous cubic phase (62:38 wt%), where the aqueous solution initially contained 3.5 wt% glucose, after 3 h (●) and 4 h (○) equilibration against pure water. The concentration is given as the wt% glucose in the aqueous solution of the cubic phase. The solid and broken lines are represent the best theoretical fit of Fick's law, giving diffusion coefficients of 1.39×10^{-10} m^2 s^{-1} and 1.47×10^{-10} m^2 s^{-1} after 3 and 4 hours, respectively. The corresponding bulk value is 6.7×10^{-10} m^2 s^{-1}. The data, obtained by holographic laser interferometry, are adopted from Mattisson et al. (Mattisson et al. 1996; Nylander et al. 1996), where also the experimental details are given. (**b**) NMR self-diffusion coefficients at 25 °C in monoolein-aqueous cubic phases containing 0–5 wt% vitamin K_1, are shown as a function of the lipid volume fraction (including vitamin K_1). The self-diffusion coefficients were measured in the cubic (both gyroid and diamond type) and in the reversed micelle, L_2, phases. Self-diffusion coefficients of monoolein (D_{MO}) (●) and vitamin K_1 (D_{VK}) (○) are shown. The lines are arbitrary fits to demonstrate the similar trends. The data are adopted from Caboi et al. (1997), where also the experimental details are given

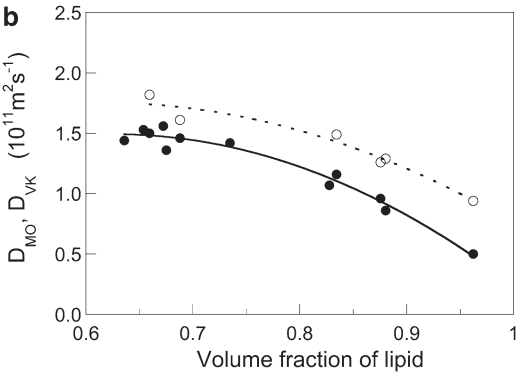

Fig. 5.21 (continued)

is certain to be affected by the dimensions of the channels and the size of the solute. Thus, electrochemical studies of the transport of cytochrome c in the monoolein-aqueous cubic phase gave values of diffusion coefficients that were about 70 times lower than the bulk values (Razumas et al. 1996b). Figure 5.21b shows the mobility of monoolein and vitamin K_1, dispersed in the lipid bilayer as the NMR self-diffusion coefficients plotted versus lipid volume fraction in the cubic phases. It is noteworthy that the mobility of the introduced vitamin K_1 follows that of monoolein, indicating complete dispersion of vitamin K_1.

The dimensions of the water channels in the bicontinuous cubic phases, which depend on the degree of swelling and type of cubic phase are in the same range as the size of proteins (cf. Barauskas et al. 2000). Furthermore, as liquid crystalline phases they are quite flexible structures. These features have triggered a number of studies, which have shown that a large range of hydrophilic proteins with molecular weights up to 590 kD can be entrapped in the aqueous cavity of the monoolein-aqueous cubic phases (Razumas et al. 1994, 1996b, b; Nylander et al. 1996; Leslie et al. 1996; Barauskas et al. 2000). The entrapped proteins have been found to be protected in the cubic phase, with retained native confirmation (Ericsson et al. 1983b; Portmann et al. 1991; Landau and Luisi 1993; Razumas et al. 1996a; Leslie et al. 1996) and some enzymes can be kept for a very long time (months in some cases), with retained activity, which is not possible in aqueous solution (Razumas et al. 1994; Nylander et al. 1996).

Spectroscopic data have revealed changes in the molecular organisation of the lipids evoked by the presence of the protein. FT-IR measurements on the monoolein—cytochrome c aqueous system showed that the presence of cytochrome c increased the conformational order of the monoolein acyl chain and caused structural rearrangements in the polar head group region (Razumas et al. 1996b). These observations are in agreement with the decrease of the monoolein packing parameter on upon incorporation of cytochrome c, which was deduced from increase in unit cell dimension of the cubic phase as determined by small angle X-ray diffraction. Here it should also be mentioned that lipid liquid crystalline sponge

phase (L3) has all the advantages from the bicontinuous cubic phase, but the water channels large and lipid matrix is more fluid than for the cubic phase (Valldeperas et al. 2016).

The cubic monoglyceride phases have also the ability to solubilise lipophilic proteins like A-gliadin from wheat (Larsson and Lindblom 1982) and bacteriorhodopsin (Landau and Rosenbusch 1996) as well as relatively large amounts of membrane lipids (Baruskas et al. 1999; Razumas et al. 1996a; Nylander et al. 1996; Gutman et al. 1984; Engblom et al. 2000) and other hydrophobic compounds of biological relevance (Caboi et al. 1997, 2001; Baruskas et al. 1999). These compounds are most probably dispersed in the lipid bilayer region of the cubic phase. The cubic phases can be used to achieve unique delivery functionalities in food systems, e.g. to solubilize functional ingredients and nutrients and to control release of flavors. Other applications in food systems can be to protect molecules from chemical degradation, or to increase the yield in Maillard reactions (Sagalowicz et al. 2006a).

Razumas et al. (1994) demonstrated that cubic monoolein-aqueous phases, containing enzymes, could be used as the biocatalytic layer in amperometric and potentiometric biosensors. Their results for biosensors, based on a variety of enzymes, show that the long-term stability decreases in the order lactate oxidase > creatinine deiminase > glucose oxidase > urease, that is basically in the order of increasing molecular weight. This implies that the enzymatic load and activity within such cubic phases are restricted by the relatively small diameter of the aqueous channels. In fact the enzymatic activity of horseradish peroxidase (HRP) included in such a phase can be increased by increasing the dimensions of the water channels and thus relaxing the confinement (Sun et al. 2014). This increase in swelling could be achieved by including varying amount of hydration-enhancing sugar ester in the lipid matrix.

Also the cubic phases of other amphiphiles like ethoxylated fatty alcohols can be used to entrap glucose oxidase, to construct a simple glucose monitor (Wallin et al. 1993). Landau and Rosenbusch demonstrated that the bicontinuous phases based on monoolein and monopalmitolein could provide matrices for the crystallisation of membrane proteins like bacteriorhodopsin (Landau and Rosenbusch 1996). They pointed out that the use of these types of cubic phase is advantageous as they provide nucleation sites, as the membrane proteins can be dissolved in the lipid bilayer. In addition they support growth by allowing lateral diffusion of the protein molecules in the membrane. Since these early studies the use of cubic phases for protein crystallization has increased tremendously (Conn et al. 2012; Caffrey 2015; Salvati Manni et al. 2015).

The bicontinuous cubic structures have by virtue of their well defined porosity also a large potential in drug delivery systems (Larsson 1994). Stable particles of lipid-aqueous cubic phases, known as cubosomes, can also be produced for this purpose (Larsson 1989, 1994, 2000; Landh 1994; Gustafsson et al. 1996, 1997). The stability of cubosomes, formed in monoolein -H_2O-based systems, and the corresponding dispersed H_{II} phase (Hexosome® particles) in the monoolein-triolein-H_2O system was found to increase in the presence of an amphiphilic block-

copolymer (polyoxamer) (Landh 1994; Gustafsson et al. 1996, 1997). Barauskas et al. have devised a method to prepare very monodispersed liquid crystalline nanoparticles (LCNP) and they found it was possible to further controlling dispersion particle size and nanostructure by varying the amphiphile concentration, the amount of charged species, and salt content (Barauskas et al. 2005b, 2009; Johnsson et al. 2006; Wadsater et al. 2014). In fact they showed that it is possible to prepare a range of different nanoparticle dispersions of self-assembled lipid mesophases with distinctive reversed cubic, hexagonal, and sponge phase structures by tuning the lipid composition and a simple, generally applicable and scalable method. Some of these structures are shown in Fig. 5.22 (Barauskas et al. 2005b). A strong correlation between the mesophase internal structure and the shape of the nanoparticles was observed. For example, monocrystalline cubic-phase nanoparticles tend to maintain the shape of the cube, hexagonal phase give the shape of a hexanon, while the highly disordered "sponge" phase structures, favor the spherical shape. (Guillot et al. 2006) identified possible internally self-assembled phases that occur in oil-loaded monoglyceride-based nanoparticles that are dispersed in water. The internal structure of these particles could be changed by changing the temperature transformating from hexosomes to emulsified micro-emulsions through micellar cubosomes (emul-

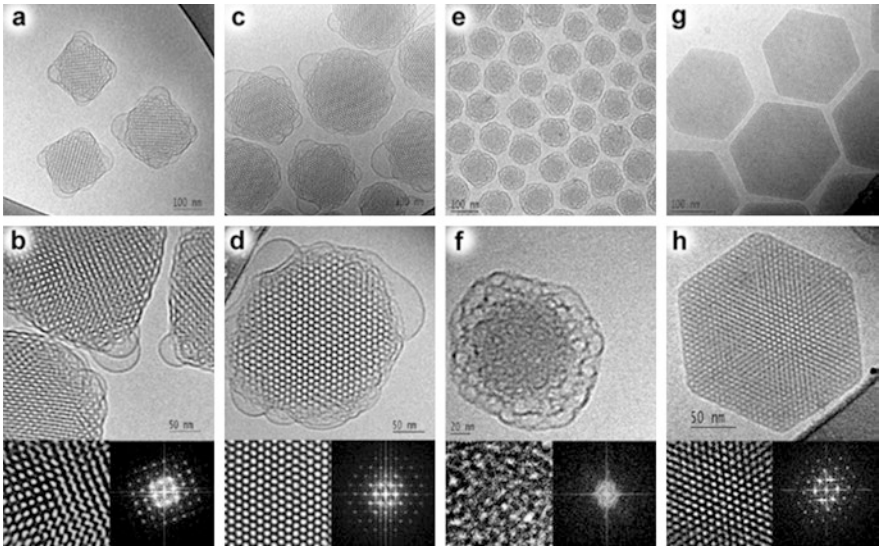

Fig. 5.22 Representative cryo-TEM micrographs of different nonlamellar lipid nanoparticles: Reversed bicontinuous cubic phase particles viewed along [001] (**a** and **b**) and [111] (**c** and **d**) directions. These dispersions were prepared at the weight ratio GMO/ F127/water) 1.88/0.12/98.0. Panels **e** and **f**: Monodisperse "sponge" phase nanoparticles prepared at the weight ratio DGMO/ GDO/P80/water) 2.13/2.13/0.74/95.0 (**e** and **f**). Reversed hexagonal monocrystalline particles made of lipids at the weight ratio DGMO/GDO/F127/water) 2.25/2.25/0.5/95.0 (**g** and **h**). Fourier transforms of magnified areas in panels **b**, **d**, **f**, and **h** show the structural periodicity of the different nanoparticles consistent with the mesophase structures indicated above. The picture is kindly provided by Justas Barauskas and further details are in (Barauskas et al. 2005b)

sified reversed discontinuous micellar cubic phase) within a narrow range of an oil/monoglycerides ratio.

Several studies on different type of dispersed liquid-crystalline nanoparticles (LCNP) have pointed on the potential of using these systems for drug delivery as well as delivery of functionality to foods (Vandoolaeghe et al. 2006; Barauskas et al. 2005a, b, 2006a, b; Sagalowicz et al. 2006a, b; Tamayo-Esquivel et al. 2006; Boyd et al. 2006; Johnsson et al. 2006; Almgren and Rangelov 2006; Angelov et al. 2006; Yaghmur et al. 2006; Worle et al. 2006; Spicer 2005a, b; Esposito et al. 2005; Monduzzi et al. 2014; Negrini et al. 2014; Nguyen et al. 2011; Zhai et al. 2015). This has been shown, with both model and in vivo studies for the drug substance propofol; a well-known anesthetic agent currently used in clinical practice in the form of a stable emulsion (Johnsson et al. 2006). The propofol-LCNP formulation shows several useful features including: higher drug-loading capacity, lower fat-load, excellent stability, modified pharmacokinetics, and an indication of increased effect duration. Interestingly it has recently been shown that it is possible to combine targeting with self-assembled lipid nanoparticles containing internal nanostructures (Zhai et al. 2015).

An interesting aspect of the interaction between liquid crystalline phases and proteins is the study of Angelova et al. (2005, 2006). They showed that supramolecular three-dimensional self-assembly of nonlamellar lipids with fragments of the protein immunoglobulin gave bicontinuous cubic phase fragmented into nanoparticles with open water channels. These so-called proteocubosomes are nanostructured open-nanochannel hierarchical fluid vehicles characterized by a cubic lattice periodicity of the lipid/protein supramolecular assembly (protein-loaded cubosomes).

5.6 Conclusion

The interaction between emulsifiers and proteins is to a large extent driven by electrostatic or hydrophobic interactions, or in many cases it is a combination of the two. Thus, it is commonly observed that ionic emulsifiers interact more strongly with proteins than nonionic ones. For emulsifiers with low water solubility, e.g., polar lipids, the interaction with proteins is largely dependent on the phase structure upon addition. The binding can, depending on the type of emulsifier, lead to stabilization of the protein structure at low-surfactant-to-protein ratios. However, an increase in surfactant concentration can induce unfolding of the protein and in some cases precipitation of the protein.

We have seen that the stability of emulsions and foams is determined by interfacial processes, which are affected by the properties of the interface as well as the interactions occurring in bulk solution. When no emulsifier/protein interactions are present, the composition of the interfacial film is determined by only the surface activity and concentration of the components. In the case of reversibility the most surface-active and/or abundant molecule dominates the interface and in the case of irreversibility the transport rate “the race for the interface” might also play a role. In

this context it has to be born in mind that proteins can change their conformations (sometimes in a time-dependent way) at the interface. This may lead to a strong interaction between the protein and the surface, and multiple interactions between neighbouring protein molecules. The latter has been found to hamper the displacement of a protein by more surface-active emulsifiers.

The presence of protein/emulsifier interactions can have pronounced impact on the interfacial behavior of the components. In cases where the emulsifier binding induces protein unfolding, exposure of hydrophobic domains of the protein, or precipitation at the interface due to charge neutralization, the surface activity of the complex is increased compared to the native protein. On the other hand, if the protein is more soluble or stabilized by the emulsifier interaction, the complex has a reduced tendency to adsorb at the interface. Precipitation of the complex in the bulk can cause loss of surface-active material and hence a decrease of the surface concentration. The emulsifier/protein interactions at interfaces can give more efficient packing and thus a higher total surface concentration. If protein/protein interactions take place at the interface, they may be disrupted by protein/emulsifier interactions.

Although emulsions and foams are stabilized by the same mechanisms, there are marked differences. First, there are profound differences between the two types of liquid interfaces: the liquid/air and the one between two condensed media. The oil/aqueous interface allows hydrophobic residues to become dissolved in and interact favorably with the oil phase, which is not possible at the air/water interface. It should be noted that unfolding of the protein induced by action of emulsifiers or by the presence of an interface generally leads to exposure of hydrophobic residues; that is, the unfolded protein is more "oil soluble" than the native one. Second, in the stabilization of foams the viscoelastic properties of the surface film as well as the thin aqueous film have large effects. This means that protein/protein interactions in protein-stabilized foams are important, and the addition of surfactants can disrupt these interactions and lead to the collapse of the foam. On the other hand, low molecular weight emulsifiers can also stabilize the foam by means of Gibbs and Marangoni effects.

Steric and/or repulsive forces are important for stabilization of emulsions. Therefore, the mixed-protein/emulsifier layer should be optimized with respect to charge and/or by segments in the surface layer protruding into the aqueous environment to give a hairy structure that will sterically stabilize the emulsion.

This chapter has shown the enormous variety in emulsifier/protein interactions that can occur in food emulsions and foams. Each protein/emulsifier combination is unique and its behavior specific when applied in a particular foam or emulsion, where other ingredients are present. However, we have demonstrated that it is possible to establish certain principles for protein/emulsifier interactions. These principles based on mechanisms at the molecular level have also to be transferred to processes of manufacturing, storage, and distribution of food products based on emulsions and foams. Apart from the stability issues, other challenges are to increase the resistance of microbial growth without excessive use of antimicrobial substances, control digestion of the product, achieve controlled release of flavors as well as design new functional ingredients based on natural products.

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Chapter 6
Physicochemical Aspects of an Emulsifier Function

Björn Bergenståhl and Patrick T. Spicer

6.1 Introduction

The characteristic property of all emulsifiers is their surface activity. Surface activity is the ability to form a surface excess at interfaces. The formation of adsorbed emulsifier layers at interfaces is displayed in a change of a range of easily observable and technically important properties:

1. The surface tension is reduced.
2. The lifetimes of bubbles are increased. Only very pure water displays a very short lifetime, a few seconds, of bubbles created by shaking. Normal water, even double distilled, usually displays a bubble lifetime of about 20–30 s.
3. The emulsifiability of oils in water is enhanced. Smaller drops with a longer lifetime are formed with less stirring.
4. The aggregation rate of dispersed particles is changed. Surface-active additives may induce or prevent flocculation of dispersions.
5. The sediment volume of settling particles is influenced. Surface additives inducing adhesion may create a loose or compact sediment.
6. Crystallization properties are changed. This may include crystallization rate and crystal shape.

This chapter aims to discuss the principal physical origin of the various functions of typical lipid food emulsifiers. Emulsifier function under very different conditions

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in various foods will be discussed. The chapter will try to show how to select emulsifiers on the basis of their fundamental properties.

6.2 Surface Activity

When an additive is added to a solution, the gain of entropy is very large at low concentrations. If the additive displays surface activity and adsorbs at an interface, the system loses entropy, which has to be balanced by a gain in free energy due to the adsorption. At very low concentrations the solubility always prevails, but when the concentration is increased, more and more of the available surfaces will be covered by the adsorbed molecules. To display surface activity, an emulsifier needs to have certain properties:

1. It has to produce a non-crystalline form[1] in contact with water.
2. It should have a low solubility in water due to a large hydrophobic part.
3. It has to interact with water through polar interactions.
4. It should have a significant molecular weight to reduce the effect of the decreased entropy when it adsorbs.
5. It has to have a reduced solubility in an oil environment due to large size and the presence of polar groups at the interface.

High-melting emulsifiers do not display surface activity when dispersed in water until a critical temperature, the Krafft temperature, has been reached. At this temperature the emulsifier solubility in the solution has reached a sufficient concentration to allow for a significant formation of adsorbed layers at the interfaces.

The presence of hydrophobic parts of the molecules increases the energy gain due to adsorption. In aqueous environments most emulsifiers tend to aggregate in micelles above a key concentration, CMC (critical micelle concentration), or to precipitate as liquid crystals. Above the aggregation concentration all properties depending on the chemical potential, for instance the interfacial tension, are more or less constant. The aggregation is mainly driven by the presence of the hydrophobic parts of the molecules (Tanford 1973).

A polar part of the molecule is necessary to avoid the formation of a separate oil phase. The type of aggregates formed during the adsorption will reflect the balance between the polar part and the hydrophobic part of the molecule.

The free-energy gain at adsorption is mainly proportional to the molecular weight, while the entropy loss due to the demixing is independent of molecular weight. Hence, small molecules like lower alcohols do not form adsorbed layers at hydrophobic surfaces in contact with water solutions, while pronounced layers are formed with additives of higher molecular weights, for instance monoglycerides. Proteins display a much higher surface activity than protein hydrolysates.

In an oil environment, solvophobic effects are absent and the adsorption has to be generated by polar interactions between the second phase and the surface-active molecule.

Table 6.1 Effects of changes in droplet interactions on the macroscopic properties of emulsions

Interaction	Stability	Sedimentation outcome
Attraction	Flocculation	Large, low density sediment volume
Repulsion	Stable	Small, dense sediment volume

The interaction between droplets is influenced when an adsorbed layer of an emulsifier covers the droplets. The change in the interaction strongly influences the macroscopic properties of the dispersions. Table 6.1 indicates the effects of droplet interactions on droplet aggregation state and the resulting sediment volumes. Flocculated droplets form open, porous aggregates that do not pack as tightly as stable particle dispersions during sedimentation, producing larger sediment volumes.

The solution properties of emulsifiers are determined for the surface activity of the emulsifiers. In addition, the ability to generate repulsive interactions is also reflected in the solution properties of emulsifiers.

6.3 Solution Properties of Emulsifiers

When water is added to a surfactant system, the solution state of the system may in principle pass through a series of aggregation structures and phases in a particular sequence. Depending on the emulsifier structure, some phases may be omitted. The sequence is: reversed, water-in-oil, micelles → reversed hexagonal phase → cubic phase → lamellar phase → hexagonal phase → normal, oil-in-water, micellar solution → molecular solution (Fontell 1978) (Fig. 6.1). The presence of certain phases like lamellar and hexagonal phase can be detected using polarized light microscopy to match the birefringent textures formed by these phases (Rosevear 1968; Laughlin 1994).

The free energy of solubilization, $\Delta G_{solubilization}$, can be described as a sum of free energy contributions in the process by the expression: emulsifier phase + water → more solubilized phase:

$$\Delta G_{\text{phase transformation}} + \Delta G_{\text{mixing}} + \Delta G_{\text{polar group/water interaction}} + \Delta G_{\text{hydrophobic}}$$

where ΔG_{mixing} is negative when changing from large aggregates to small aggregates like micelles and molecular solutions.

$\Delta G_{hydrophobic}$ is positive and equal to the product of the area per molecule at the interface, $A_{hydrocarbon/water}$, and the oil-water interfacial tension, $\tilde{u}FE;_{hydrocarbon/water.}$ The hydrophobic effect is the driving force for the aggregation and gives the upper limit of the molecular solubility for amphiphilic molecules: the CMC or critical micelle concentration.

$\Delta G_{polar\ group/water}$ is negative. This term consists mainly of the work released when more water allows a larger separation between repelling aggregates or molecules:

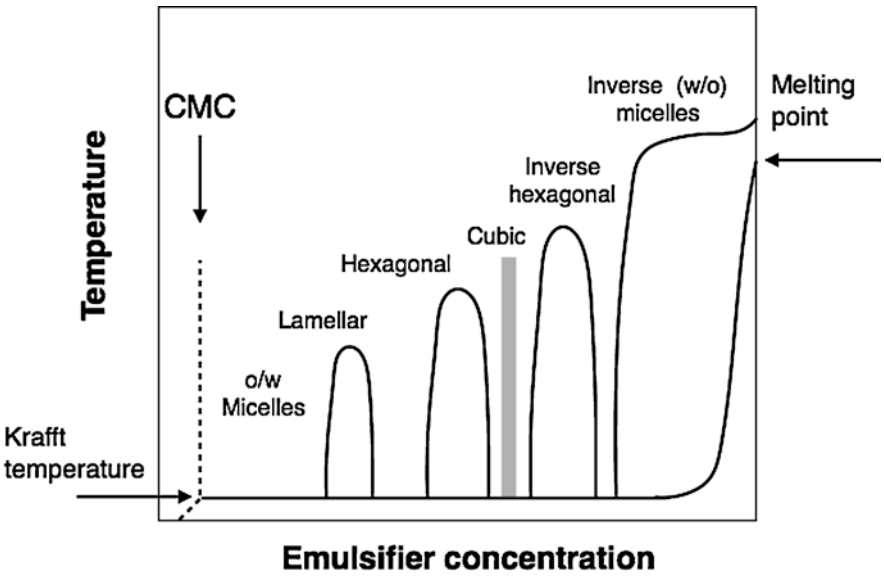


Fig. 6.1 A typical sequence of liquid-crystalline phases and solution phases formed in an aqueous emulsifier system. (Modified from Fontell 1978)

$$\Delta G_{\text{polar group/water}} = \sum_{\text{all neighbors}} \left\{ \int_{\text{next aggregate}}^{\text{aggregate}} F(l)\,dl \right\}$$

where l is the average distance between the polar groups, also called head groups, and $F(l)$ is the interaction force.

The area per molecule in the aggregates is found from the balance between the interfacial tension of the oil/water interface and of the space needed for the polar group itself in addition to any space generated by repulsive interactions between the emulsifier head groups at the interface.

The area per molecule expands in the series ^{A}reversed micelles $<^{A}$reversed hexagonal $<^{A}$cubic $<^{A}$lamellar $<^{A}$hexagonal $<^{A}$micelles.

At a specific ratio of water and emulsifier, the system's tendency is to obtain aggregates as small as possible to maximize the ΔG_{mixing} and the $\Delta G_{\text{polar group/water}}$. The lower limit in aggregate size is given by the onset of increased hydrophobic contact with the exposed hydrocarbon/water interface.

The interesting result of this exercise is that the interfacial area per molecule is to a large extent a measure of the ability to generate repulsive interactions.

In the solubilization sequence, reversed aggregates → lamellar phase → hexagonal phase → micellar solution → molecular solution, the area per molecule of the surfactant/water interface increases. Depending on the packing constraints given by the hydrophobic moiety in the aggregates, the range of the repulsive interaction on the polar side of the molecule, and the molecular weight, this process has to proceed

more rapidly or more slowly (Israelachvili et al. 1976, 1977). Hence, the packing constraints of the hydrocarbon chain are an important link between proper- ties and aggregation.

The ratio of the actual area A, as it is created by the repulsive interactions, to the theoretical area of a saturated hydrocarbon chain, A_0 = 23 Å^2 enforces different aggregate geometries (Israelachvili et al. 1976, 1977) based on the most efficient packing of a given molecule into an aggregate and can be described using the different ratios of volume to area for common aggregates, as shown in Table 6.2. Spherical micelles form when the polar group area dominates molecular geometry, as conical shapes pack well into a sphere. Similarly, increasingly cylindrical geometries pack into flatter structures as the packing parameter approaches unity.

The successive solvation of surfactants in Table 6.2 corresponds to a successive change into aggregates because of more long-range interactions. If there is an upper limit for the repulsion, the solvation series is terminated at that stage. Hence, the maximum solvated aggregate formed in a surplus of water is a measure of the ability of the emulsifier to generate repulsive interactions.

Table 6.2 The geometries of different aggregates

Area volume	Packing constraint[a] (A_0 = 23 Å^2 for a saturated hydrocarbon tail)
Sphere (micellar solution)	
$\frac{2\pi r^2}{(4/3)\pi r^3} = \frac{2}{r}$	$\frac{3 \times V_{\text{hydrophob}}}{r_{\text{hydrophob}}} = 3A_0$
Rods (hexagonal phase)	
$\frac{2r\pi l}{\pi \times r^2 l} = \frac{2}{r}$	$\frac{2 \times V_{\text{hydrophob}}}{r_{\text{hydrophob}}} = 2A_0$
Bilayers (lamellar phase)	
$\frac{2l^2}{2rl^2} = \frac{1}{r}$	$\frac{1 \times V_{\text{hydrophob}}}{r_{\text{hydrophob}}} = A_0$
Reversed rods (reversed hexagonal phase)	
$\frac{2\pi r_{aq} l}{\pi l\left[\left(r + r_{aq}\right)^2 - r_{aq}^2\right]} = \frac{2}{r\left(1 + \sqrt{1 + 1/\phi_{aq}}\right)}$	$<A_0$

r = radius of the aggregate, usually limited by the length of molecule
l = a fictitious length of the aggregate
$V_{\text{hydrophob}}$ = volume of the hydrophobic part of the molecule
$r_{\text{hydrophob}}$= the maximum length of the hydrophobic moiety
A_0 = area of a cylindrical packing of the hydrophobic moiety (= $V_{\text{hydrophob}}/r_{\text{hydrophob}}$ or 23 Å^2 per hydrocarbon chain)
A = area per molecule at an average water/amphiphilic interface
[a]The packing constraint is here defined as the necessary cross section of an amphiphilic molecule in the aggregate at the oil/water interface. This definition is A_0/packing parameter according to Israelachvili et al. (1992, 1976, 1977)

The area of the molecule is a measure of the interaction when water is present, and may be generalized as the hydrophilicity of the molecule. The spatial requirement of the hydrophobic part of the molecule is of course a measure of the hydrophobicity of the molecule. Consequently, there is a close link with the classical view of emulsifiers as molecules with a balance between the hydrophobic and the hydrophilic properties, as they are expressed in the HLB numbers, proposed by Griffin (1949, 1979).

6.4 The Use of Phase Diagrams to Understand Emulsifier Properties

Friberg and coworkers (Wilton and Friberg 1971; Friberg and Mandell 1970a; b; Friberg and Rydhag 1971; Friberg and Wilton 1970; Rydhag 1979; Rydhag and Wilton 1981; Friberg et al. 1969; Friberg 1971) have investigated phase diagrams and emulsion stability extensively. They concluded that the optimum composition for a stable emulsion should be that at which the lamellar phase, the oil phase, and the water phase are in equilibrium in the corresponding phase diagram (Fig. 6.2).

The relation between the formation of lamellar phases and emulsion stability is basically of an empirical nature. The emulsifiability is enhanced at certain compositions (Friberg and Mandell 1970b; Friberg and Rydhag 1971; Friberg and Wilton 1970), and the formation of crystalline phases corresponds to an observed destabilization (Wilton and Friberg 1971). The formation of multilayers around the emulsion droplets under certain conditions has also been shown (Friberg 1990).

It was suggested that the formation of a multilayer of a lamellar liquid-crystalline phase coating the droplet surface reduces the van der Waal's attraction and that this was an important contribution to the observed effects in the emulsification experiments (Friberg 1971). However, this explanation is not a useful general explanation since the emulsifier concentration in optimized food emulsions rarely is high enough to allow for multilayer adsorption (Walstra 1988; Dickinson 1986). Obviously, this observation is contradictive to the need for a separate phase of liquid-crystalline material around the droplet. However, a correlation between the presence of, or the possibility to form, liquid-crystalline phases and emulsion stability is still experimentally observed in several systems. More generally, to stabilize a dispersion, the emulsifier should:

1. Contribute to the repulsive interactions between the droplets
2. Contribute to the interfacial viscosity
3. Be well anchored to the interface

These properties are reflected in the formation of various liquid-crystalline phases, such as lamellar bilayers (Table 6.3). These aspects are illustrated by a few examples.

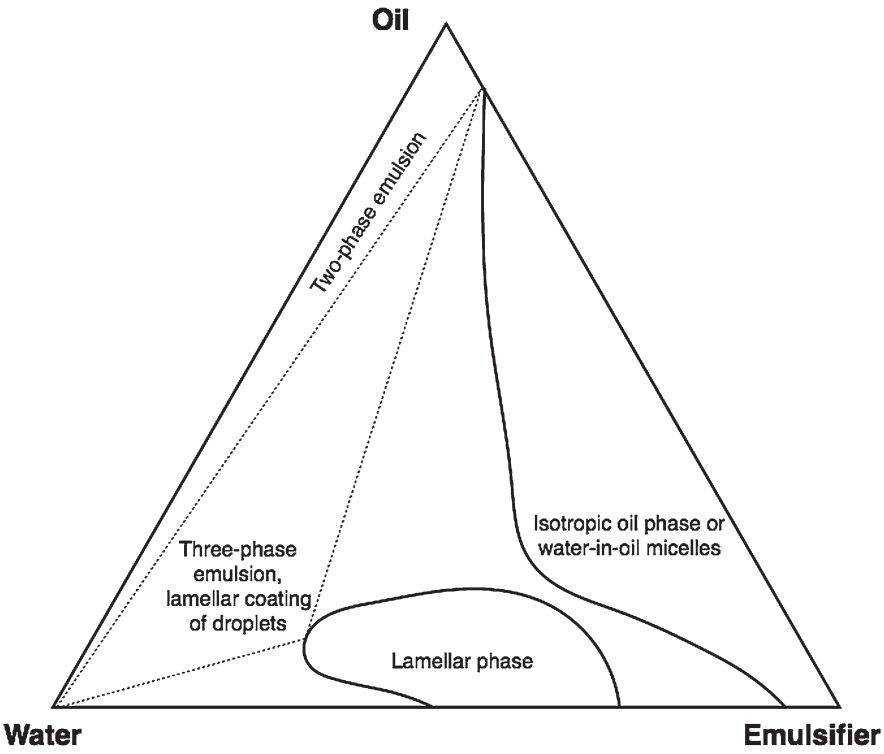


Fig. 6.2 Schematic drawing of the ternary structure map for an emulsion system with an emulsifier forming lamellar liquid-crystalline phase. A coating of the droplets can form at higher emulsifier levels, improving stability. (Modified from Friberg 2006)

Table 6.3 The relation between the function of an emulsifier to stabilize an emulsion and its ability to form various aggregation structures

Stabilizing property aggregates	Micelles	Bilayers	Reversed
	Water-continuous emulsions		
Repulsive interactions	Optimal	Intermediate	Weak
Interfacial viscosity	Weak Optimal	Weak	
Anchoring	Too water-soluble	Optimal	Acceptable
	Oil-continuous emulsions		
Repulsive interactions	Weak	Intermediate	Optimal
Interfacial viscosity	Weak	Optimal	Weak
Anchoring	Acceptable	Optimal	Too oil-soluble

There is also utility to the formation of cubic phases (Rodriguez et al. 2000) as their solid-like rheology prevents emulsion coalescence and sedimentation by gelling the emulsion, but little work has been done to study their performance at much lower phase volumes.

6.5 Examples of the Relation Between Phase Diagrams and Emulsion Stability

6.5.1 Monoglycerides

A technical monoglyceride at room temperature remains in a nonhydrated crystalline phase, or β phase, in equilibrium with a surplus of water. Above 40 °C, the monoglyceride takes up water and a lamellar phase is formed (Wilton and Friberg 1971). The lamellar phase coexists with a surplus of water (no micelles are formed). When the lamellar phase is cooled, a semicrystalline phase, termed "α phase," is formed. This phase is metastable below 30°C and converts only slowly into an aqueous and a β phase.

The swelling of the lamellar and α/gel phases indicates the existence of a strong repulsive hydration force. This force has been measured by the osmotic stress technique (Fig. 6.3). In contrast, no hydration force strong enough to separate the bilayers is present in the β phase. The hydration force between emulsion droplets coated with this emulsifier depends on the liquid-crystalline state of the adsorbed emulsifier film in the same way. This explains why monoglycerides appearing in the β form are inactive as emulsifiers, and why a monoglyceride-stabilized emulsion rapidly destabilizes when the monoglyceride converts from lamellar or α into β phase (Wilton and Friberg 1971). In technical systems, it is important that the conversion of α phase into β phase is delayed. An α phase can be stabilized by the presence of ionic charges, as with soap (Larsson and Krog 1973), or by a wide distribution of the fatty acid-chain lengths. The solution properties of a range of food emulsifiers are summarized in Table 6.4.

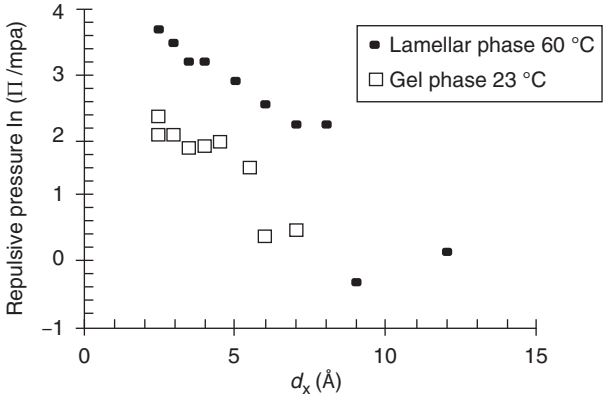


Fig. 6.3 The hydration repulsion between bilayers of monopalmitin in the liquid-crystalline and gel states. (Redrawn from Pezron et al. 1991)

Table 6.4 Formation of liquid-crystalline phases by lipid emulsifiers

Emulsifier	Fatty acid	Liquid-crystalline phases formed at	Upper swelling limit (at 25 °C)	
Monoglycerides: Distilled saturated	C18–16	Lamellar phase at 50 °C	50%	Krog (1990)
		Cubic at 70°C		
Distilled unsaturated	C18:1–2	Cubic < 20 °C	35%	Krog (1990)
		Reversed hexagonal at 55 °C		
Monoolein	C18:1	Cubic < 20 °C	40%	Krog (1990)
		Reversed hexagonal at 90 °C		
Tetraglycerolesters:				
Tetraglycerol monolaurin	C12	Lamellar < 20 °C	55%	Krog (1990)[a]
		Fluid isotropic 40 °C		
Organic acid esters:				
Diacetyl tartaric acid monoglyccridc ester	C16–18	Lamellar 45 °C	55%	Krog (1990)
Sodium steraoyl lactylatc:				
pH 5	C18	Reversed hexagonal at 45 °C	40%	Krog (1990)
pH 7	C18	Lamellar at 42 °C	60%	Krog (1990)
Sorbitan eslers:				
Polyoxyethylene (20) sorbitan monooleate	C18:1	Hexagonal phase (up to 30 °C) and micellar solution	—	Hall, Pethica (1967)
Polyoxyethylene (20) sorbitan monostearate	C18	Hexagonal phase (30–50 °C) and micellar solution above 30 °C	—	Hall, Pethica (1967)
Sorbitan stearate	C18	Lamellar above 50 °C	—	Hall, Pethica (1967)

[a]The data are extracted from a review of several original sources

6.5.2 Lecithins

Lecithin is one of the most commonly used food emulsifiers, and its popularity is expected given its natural origin. Technical lecithins, usually soybean lecithin, are always natural mixtures of various phospholipids. The most frequent one is phosphatidylcholine, PC. The second is phosphatidylethanolamine, PE. Phosphatidylinositol, PI, and phosphatidic acid, PA, are usually present at intermediate levels, and phosphatidyl serine, PS, lysophosphatides, LPC and LPE, etc., at low levels. Nonphosphatides such as steroids, vitamin E, and free fatty acids are usually also present in technical products. The properties of lecithins reflect some type of average

properties of the mixture. This section will first describe the characteristic properties of the most common phosphatides and then discuss the properties of various mixtures.

6.5.3 *Phosphatidylcholine*

The phase diagram of a typical unsaturated phosphatidylcholine is displayed in Fig. 6.4. The phase diagram is characterized by a large swelling lamellar phase. Saturated phosphatidylcholines have a phase transition temperature up to about 40 °C, whereas the corresponding temperature for unsaturated lecithins is well below 0 °C. The phase diagram of soybean PC is described in Bergenståhl and Fontell (1983) and is rather similar to the phase diagram of dioleoyl PC.

6.5.4 *Phosphatidylethanolamine*

Phosphatidylethanolamine is less hydrophilic than PC. The saturated ethanolamines form lamellar phases that swell less than the corresponding PC species. The phase transition temperature is about 10–40 °C above the corresponding temperature of the phosphatidylcholine (Fig. 6.5). The more limited ability of the molecule to create long-range repulsive interactions, and thereby to occupy a large interfacial area, is displayed in the tendency to form reversed hexagonal phase with unsaturated PE species, as shown in Table 6.5.

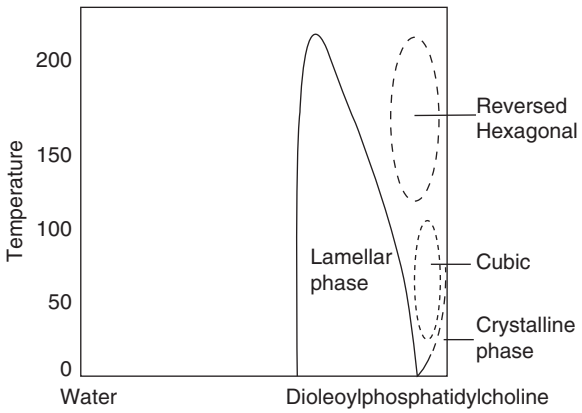


Fig. 6.4 The Phase diagram of water and dioleoylphosphatidylcholine (From Bergenståhl and Stenius 1987)

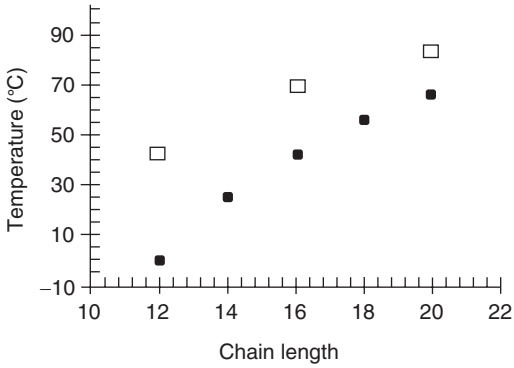


Fig. 6.5 The main transition temperature for phosphatidylcholine (open square) and phosphatidylethanolamine (filled) as a function of chain length. Sources in Table 6.4

Table 6.5 The formation of liquid-crystalline phases by various phospholipids

Phospholipid	Fatty acids	Liquid-crystalline phases formed at	Upper swelling limit (at 25 °C)	
Phosphatidylcholine:				
Distearoyl	C18	Lamellar phase at 55 °C	—	Small (1986)[a]
Dipalmitoyl	C16	Lamellar phase at 41 °C	36%	Insko & Matsui (1978)
Dimyrisloyl	C14	Lamellar phase at 23 °C	40%	Janiak et al. (1978)
Dioleoyl	C18:1	Lamellar below 0 °C	42%	Bergenstähl & Forteil (1987)
Egg PC	C16–18:1	Lamellar at 2 °C	44%	Small (1986)
Soybean PC	C18:1–2	Lamellar below 0 °C	35%	Bergenstähl & Forteil (1987)
Phosphatidyletanoleamine:				
Dipalmitoyl	C16	Lamellar phase at 68 °C	20%	Caffrey (1985)
		Reversed hexagonal at 84 °C		
Dioleoyl	C18:1	Lamellar below 0 °C	20%	Gawrish et al. (1992)
		Reversed hexagonal at 5 °C		
Soybean PE	C18	1–2[b] Reversed hexagonal above 0 °C	30%	Bergenstähl (1991)
Phosphatidylinositol:				
Soybean PI	C18:1–2[b]	Lamellar below 0 °C	Unlimited	Bergenstähl (1991)
				Söderberg (1990)
Phosphatidic acid:				
Dioleoyl	C18:1	Lamellar below 0 °C	Unlimited	Lindblom et al. (1991)
Lyso PG:				
Palmitoyl	C16	Micellar solution below 0 °C	Unlimited	Eriksson et al. (1987)

[a]The data are extracted from a review of several original sources
[b]Mainly

6.5.5 Phosphatidylinositol

The phase diagram of soybean PI and water has been determined by Bergenståhl (1991) and by Söderberg (1990). The diagram is characterized by a large lamellar phase with an unlimited swelling. The liquid-crystalline phase is formed below room temperature.

6.5.6 Phosphatidic Acid

The phase diagram of the sodium salt of dioleoylphosphatidic acid has been determined by Lindblom et al. (1991). The phase diagram is characterized by a lamellar phase that transforms to a reversed hexagonal phase at about 30% of water. This transformation occurs although there is an ionic charge on the molecules and despite their small head group. A possible explanation, supported by evidence from NMR measurements, is that this is due to ion condensation.

6.5.7 Lysophosphatides

The phase diagrams of a series of different lysophosphatides have been investigated by Arvidsson et al. (1985). Lysophosphatidylcholine has the same hydrophilic polar group as the ordinary PC but only one of the two fatty acids. This reduces the volume demand of the aggregate, and the packing parameter allows for the formation of micelles and hexagonal phases.

6.5.8 The Properties of Mixtures of Phosphatides

Technical phosphatides are always mixtures. Their properties reflect some type of average that the mixture develops. One way to investigate this is to determine the type of liquid-crystalline phase that develops when different phosphatides are allowed to interact together with water. Figure 6.6 shows the phase diagram of dioleoyl PC and dioleoyl PE in 40% water (Eriksson et al. 1985). The figure shows that a lamellar phase is formed when the system contains mainly PC, but that around 60% PE non-lamellar, hexagonal and cubic phases start to form. This change is enhanced at high temperatures.

The more highly unsaturated soybean PE and soybean PC display a similar aggregation pattern, but the temperature at which the system changes from lamellar to non-lamellar phases is lower (Fig. 6.7), and the phase diagram is dominated by the hydrophobic properties of the PE up to fairly high concentrations of PC.

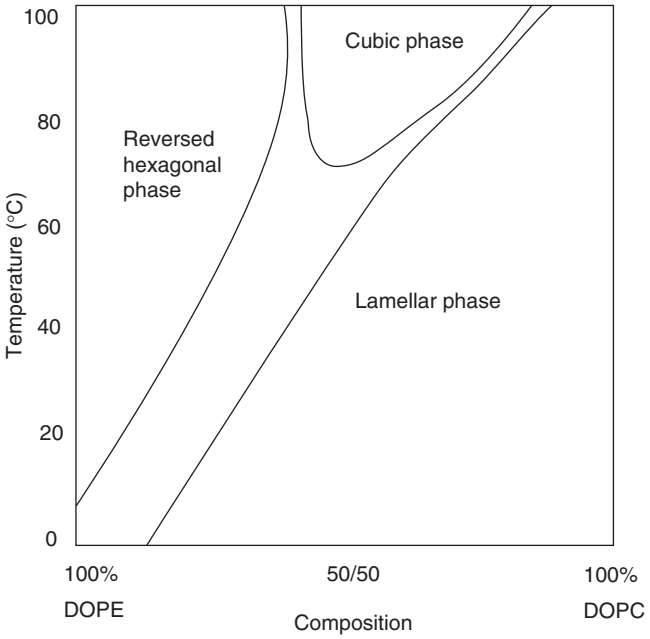


Fig. 6.6 The phase diagram of dioleoyl PC and dioleoyl PE with 40% water. (Redrawn from Eriksson et al. 1985)

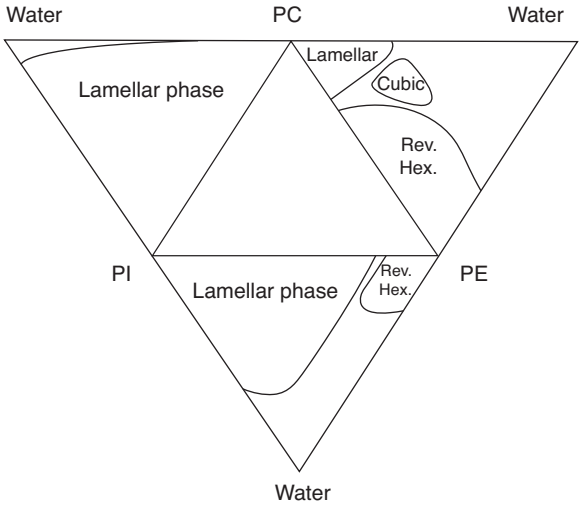


Fig. 6.7 The phase diagram of soybean PC, soybean PE, and water; of soybean PC, soybean PI, and water; and of soybean PI, soybean PE, and water. (Redrawn from Bergenståhl 1991) The cubic phase was not included in the original drawing, but it is a possible interpretation of the x-ray peaks included in the paper. It is also supported by the data from the study by Eriksson et al. (1985)

A mixture of PI and PC displays the extreme swelling properties of ionically charged emulsifiers at an early stage. This was indeed also expected since a similar pattern was observed when a small amount of ionically charged detergents was added to the lamellar phase formed by monoglycerides (Larsson and Krog 1973). When PI and PE are mixed, the properties of the mixture are dominated by the hydrophilic PI up to quite a high PE:PI ratio.

A preliminary conclusion from this work is that the properties of phosphatide mixtures are determined by the ratio of anionic (particularly PI) phosphatides to PE rather than by the PC:PE ratio.

Technical soybean lecithin contains a mixture of different phospholipids (Rydhag 1979). In most cases, the weakly hydrophilic phosphatidylethanolamine dominates, and this type of lecithin is suitable for inverse emulsions such as in margarine. More hydrophilic soybean lecithins suitable for oil-in-water emulsions are obtained by partial hydrolysis to form lysolecithins. It is also possible to increase the effective hydrophilicity of the PE by making the polar head group larger through acetylation.

6.6 Some Ways to Classify Emulsifiers

A common problem in industrial development work is the choice of suitable surfactants to obtain the desired results. In the literature a number of different methods of making a fast preliminary selection of suitable emulsifiers have been proposed. The most common methods and concepts are discussed here and are compared with the function of the emulsifier in the emulsion.

6.6.1 The Solubility Concept

One of the first ideas, proposed by Bancroft (1913), was that the solubility of the emulsifier determines the type of emulsion that is formed. An oil-soluble emulsifier will create an oil-continuous emulsion, and a water-soluble emulsifier will turn the emulsion into a water-continuous one. This is true for low molecular weight emulsifiers with a high solubility, usually in micellar aggregates, but it is also valid for polymers. However, most likely, the concept can also, to some extent, be expanded to include emulsifiers with just a dispersibility in either one of the phases (for instance lecithin). Experience in this direction is exemplified in Table 6.6. However, the Bancroft rule provides us with the only very general directions. To proceed further we need to rank emulsifiers more quantitatively.

Table 6.6 Emulsiflability compared with solubility according to the Bancroft rule (Östberg et al., 1995)

Emulsifier	Solubility/dispersibility	Type of emulsion
Sorbitan esters (Span)	Oil-soluble	Oil-continuous
Ethoxylated sorbitan esters (Tween)	Water-soluble	Water-continuous
Hydrophobic lecithin (normal technical lecithin)	Oil-dispersible	Oil-continuous
Hydrophilic lecithin (high LPC or low PE)	Water-dispersible	Water-continuous
Proteins	Water-soluble	Water-continuous
Fat crystals	Oil-dispersible	Oil-continuous

6.6.2 The Phase Inversion Concept

Ethoxylated surfactants have a tendency toward declining hydrophilicity with increasing temperature. This leads to a change from water solubility at low temperatures to oil solubility at higher temperatures. According to the Bancroft rule, this will result in a given system switching from being water-continuous to being oil-continuous. The hydrophilicity can be viewed as a property that is gradually lost with increasing temperature. The distance from the break even point, the phase-inversion temperature, or PIT, is then a measure of the strength of the hydrophilicity. Shinoda claims that the best stability of such an oil-in-water emulsion is obtained at 30 °C below the PIT and for a water-in-oil emulsion at about 20 °C above the PIT. However, the lowest interfacial tension, and the smallest droplet volume is obtained directly after homogenization by shaking at the PIT. Consequently, Shinoda suggests that the emulsifier should be chosen so that the emulsification can be performed at a PIT about 20–30 °C above the final storage temperature (Shinoda and Saito 1968). The emulsion can then be cooled and the increased interfacial tension stabilizes the small sizes obtained earlier.

Shinoda and coworkers (Shinoda and Saito 1968; Shinoda and Kunieda 1983; Kunieda and Ishikawa 1985, reviewed in Shinoda and Friberg 1986) have worked according to this concept and characterized a number of different ethoxylated emulsifiers in combination with various solvents. They then found that the PIT depends not only on the number of ethoxylate groups but also on the oil phase, indicating the importance of the solubility properties for the stability.

Emulsification experiments performed with a range of different oil-to-water ratios show that the emulsion type is determined mainly by the emulsifier properties and is for many systems, with pure solvents, very insensitive to the phase ratio (Shinoda and Friberg 1986).

It is obvious that this says a lot about the properties of ethoxylated surfactants but its applicability to food emulsions is very limited for two main reasons:

1. The concept is based on strongly temperature-dependent properties of the emulsifiers. This excludes ionic emulsifiers (less important for the food industry), and it also excludes the most commonly used polyhydroxy and nonionic zwitterionic emulsifiers as they display a very weak temperature dependence of their hydrophilicity.

2. The solvent properties are important in the PIT concept. However, food emulsions are made almost solely from triglyceride oils and water that will behave differently due to the large molecular weight of the oil molecules.

6.6.3 The HLB (Hydrophilic/Lipophilic Balance) Concept

Emulsifiers are molecules with a duality in their properties. The balance between the hydrophobic and hydrophilic properties of the molecules should then determine the performance and the type of emulsion formed. If the emulsifier is changed from hydrophobic to hydrophilic, the emulsion formed changes from oil-continuous to water-continuous. The balance of the emulsifier is recorded as a number, the HLB value. When this concept was introduced by Griffin (1949), the HLB value of unknown emulsifiers was determined by comparing the emulsification properties in a predetermined system of a mixture of hydrophobic and hydrophilic emulsifiers with a predefined HLB number.

The important development of the HLB system came when the group contribution system was constructed by Davies (1957), and it became possible to estimate an HLB value of an unknown emulsifier from the molecular formula (Table 6.7).

The advantage of the HLB concept is that it makes it possible to characterize numerous emulsifiers and emulsifier blends. It is usually assumed that it is possible to calculate an average HLB value from the w/w composition. Large tables of data for commercial emulsifiers are also available.

The limitation of the HLB value is that it provides a rather one-dimensional description of the properties, omitting molecular weight and temperature dependence. It is also difficult to calculate useful HLB values for several important food emulsifiers, for instance phospholipids. The HLB values also do not include the important crystallization properties of monoglycerides and modified monoglycerides.

Table 6.7 Calculation of HLB numbers according to Davies (1957)

Group	Group contribution
Carboxylic acid soap	21.2
Sorbitan ester	6.8
Glyceryl ester	5.25
Ester	2.4
Carboxylic acid	2.1
Alcohol	1.9
Ether	1.3
EO group	0.33
CH_3 CH_2, CH	–0.475

The table is modified according to Davies (1957). HLB = 7 + Σ group contributions (*From Bergenståhl and Claesson, 1990*)

Aggregate	Shape	Character in solution	HLB	A/A_0
Micelles		Clear solution	> 13	> 2
Lamellar phase		White dispersion Milky appearance	7–10	1–2
Reversed aggregates		Lumps of emulsifier	< 7	< 1

Fig. 6.8 A comparison between molecular aggregation, solution characteristics, A/A_0, and the packing parameter. (Modified from Bergenståhl and Claesson 1990)

6.6.4 *A Comparison Between the HLB and the Geometry of the Molecule*

There is an obvious analogy between the idea of a hydrophilic lipophilic balance and that of the balance in the molecules appearing in the packing parameters of different association structures (Fig. 6.8). Griffin (1979) has also suggested a relationship between various solution properties and the HLB number. Transforming these descriptions into various aggregation structures, a clear relation between the molecular packing and the HLB value is obtained.

This result shows that the ability to form liquid-crystalline phases corresponds to the traditional HLB characterization of the emulsifiers.

6.6.5 *The Role of the Emulsifier in Homogenization*

The discussion so far has been dealing mainly with the situation when droplets are protected by a layer of emulsifier. However, the emulsifiers also have a crucial role during the emulsification that usually is included in all empirical tests that are the bases for the rules.

When an emulsion is created from a large and homogeneous oil phase, the emulsifier should support two different processes: the formation of new droplets and protection against recoalescence. The emulsifier acts according to both static and dynamic, diffusion-limited, interactions (Walstra 1983) (Table 6.8).

Table 6.8 The role of the emulsifiers during the formation of emulsions

	Static	Dynamic
Destabilize the interfaces	Interfacial tension	Diffusion to and across the interfaces
Stabilize the droplets	Repulsive surface forces	Diffusion to the interfaces

The principal role of the interfacial tension is obvious. The presence of emulsifiers lowers the interfacial tension from about 30 mN/m for a triglyceride/water system to between 1 and 10 mN/m. Nonionic emulsifiers close to the PIT create densely packed interfaces with very low interfacial tensions. However, the effects of the interfacial tension itself are not very large. Walstra (1983) has shown that the droplet size is only weakly dependent on the interfacial tension.

During the homogenization, new interfaces are formed. The emulsifiers have to diffuse to the interfaces to lower the interfacial tension during the events when the droplets are formed. This process must be rapid to be successful, as rapid as the time scale for the formation of the droplets. For geometrical reasons, the diffusion from the surrounding phase of the droplet is much more rapid than the diffusion from the internal liquid. This is one important contribution to the validity of the solution rules (Bancroft, PIT, HLB, and phase diagrams).

During the homogenization, the water-soluble substances in the oil phase diffuse over to the water phase. These types of diffusion across the interfaces create disturbances that contribute to the emulsification. In many systems, this effect gives an increased efficiency if the emulsifier is added to the oil phase before the emulsification. For dispersible emulsifiers (phospholipids) there are also other reasons why it is more efficient to add the emulsifier to the oil phase instead of the water phase. During the homogenization, phospholipids tend to form stable liposomal dispersion in competition with the emulsification of the oil phase. Westesen has indeed observed that a significant fraction of the phospholipids in a commercial phospholipid emulsion for paranteral use is lost in liposomal aggregates (Westesen and Wehler 1992).

Emulsification involves an intensive application of fluid shear stress. The shear stress by itself causes a high frequency of recoalescence events. If the emulsification is to be successful the formed droplets have to be protected from recoalescing. The repulsive interactions generated by adsorbed emulsifiers create a static protection.

Hydrodynamic interactions are crucial to the result of a collision due to shear. Hydrodynamic interactions depend on the existence of an interfacial viscosity and elasticity. During the collision event, the interface close to the approaching droplet is depleted of emulsifiers due to the local flow. The surfactant-depleted zone will then have a higher interfacial tension than the surrounding emulsifier-covered droplet surface. This leads to surface diffusion in the direction opposite to the liquid flow and produces a hydrodynamic resistance. If the emulsifier is oil-soluble, emulsifier from the internal part of the droplet will diffuse to the depleted area and thereby reduce the hydrodynamic protection of the droplet.

The discussion in this section has been very qualitative, but an important point is that the emulsifiers contribute to the emulsification as well as to the stabilization.

The role of the emulsifier for the stabilization is usually difficult to identify in the simple type of shaking experiments that are the main background to the HLB, the PIT, and the phase diagram concepts. This type of simple, and thereby efficient, experiment provides information about both the emulsifiability and the stability with a certain emulsifier.

6.7 The Emulsifier Surface

The ability of various food emulsifiers to generate adsorbed layers influencing the interparticle interactions has been discussed. The type and magnitude depend on the composition of the surface generated from the adsorption process. Foods usually are complex mixtures. They may contain both low molecular weight surface-active lipids and a versatile range of more or less surface-active proteins and polysaccharides. The actual chemical composition of the emulsion droplet surface is then the key factor that determines most of the surface interactions.

In systems containing several surface-active components, three types of adsorbed layers can be identified based on how the layers are formed. In reality, the differences between the three adsorption structures discussed below are not sharp, but this simplified description can provide a basis for further discussion of the properties of complex systems.

1. *Competitive adsorption*. A monolayer containing one predominant type of molecule at the interface builds up through competition with other less surface-active components that may be replaced in the interface.
2. *Associative adsorption*. An adsorbed layer containing a mixture of several different surface-active components is formed.
3. *Layer adsorption*. One component adsorbs on top of another or itself.

6.7.1 Competitive Adsorption

In a system with several surface-active components, a homogeneous monolayer is formed by the most surface-active component. The adsorption depends on the driving force for adsorption, mainly the hydrophobic interaction. Hence, from a mixture of two emulsifiers, the most hydrophobic emulsifier will have the strongest affinity for the interface. A consequence is that under competitive adsorption the component with the lowest water solubility, e.g., the lowest critical micelle concentration (Kronberg 1983), will dominate the interface.

The character of the adsorbed layer, for instance its ability to generate repulsive interactions, is determined by the dominating compound. The structure of the layer depends on the geometrical shape of the molecules and on lateral interactions between the molecules in the layer. Nonionic surfactants may form very dense

layers due to head-group attraction. Ionic surfactants are able to form extremely loose layers due to inter-head-group repulsion.

An interesting experimental observation in agreement with this relation is that the concentration of emulsifier necessary to obtain an emulsion is much lower for ionic emulsifiers than for nonionic emulsifiers.

In a series of emulsions, we have studied the efficiency of the emulsification (Östberg et al. 1995) by droplet size measurements after homogenization. The results show that for several emulsifiers very small droplets are obtained, about 0.2–0.4 μm. The size obtained depends on the concentration of emulsifier. The nonionic emulsifiers lead to a constant droplet size down to a critical concentration, below which the ability to form emulsions is strongly reduced. The critical concentration can be compared with the thickness of the emulsifier layer on the emulsion droplet. The apparent thickness of the emulsifier layer can be estimated from the droplet size and the concentration of emulsifier on the dispersed phase, if we assume that all emulsifier is adsorbed to the interface. The apparent thickness gives the upper limit for the absorbed layer rather than the correct value:

$$\text{Thickness of emulsifer layer} = \frac{\text{volume of emulsifier}}{\text{emulsion droplet area}}$$

$$\delta = \frac{c_{em} V_{\text{emulsion droplet}}}{A_{\text{emulsion droplet}}} = \frac{c_{em} \pi d^3 / 6}{\pi d^2} = \frac{c_{em} d}{6}$$

where c_{cm} is the emulsifier concentration (v/v) in the dispersed phase. The critical thickness, the thickness of the emulsifier layer at the critical concentration, can be compared with the size of the molecule. The results show a thickness of about 60% of the theoretical length of the molecule for nonionic emulsifiers. Hydrophobic emulsifiers are less efficient during the emulsification and give very high values of the apparent thickness. The properties of the ionic emulsifiers are different as they are able to form emulsions down to extremely low concentrations corresponding to very low surface concentrations and very thin layers.

6.7.2 Associative Adsorption

In associative adsorption, a mixed adsorption layer is formed at an interface. The interfacial properties displayed are then some sort of average properties.

A typical associative system may be a long alcohol, e.g., decanol and charged surfactants like soaps. The alcohol acts as a spacer between the charged groups, which decreases the head-group repulsion within the layer and reduces the surface energy. This increases the adsorption and enhances the surface activity. Similarly, a lamellar phase is formed in the corresponding three-component phase diagram: water/sodium caprylate/decanol (Fontell et al. 1968). Mixed layers are commonly formed due to associative adsorption with natural and technical emulsifier blends.

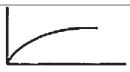
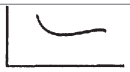
This is also a necessary requirement of the common assumption that an average HLB value should describe the properties of an emulsifier blend (Davies 1957). A common system assumed to act in this way is a mixture of sorbitan esters and ethoxylated sorbitan esters where the smaller sorbitan esters can use the space between the bulky ethoxylated esters (Boyd et al. 1976).

In the case of associative adsorption, both components are expected to be present in the surface. If this situation is to be stable, the adsorption of the second component should be either enhanced by the presence of the first component or at least not influenced by it.

6.7.3 *Layered Adsorption*

Adsorption in layers is possible when different classes of surface-active components are present in a mixture. See Table 6.9. The two components must be very different in character to give a structure with a layered character rather than a mixed

Table 6.9 The apparent emulsifier layers for various emulsifiers estimated from the earlier equation for film thickness

Emulsifier conc. (%)[a]	Radius (μm)[b]	Emulsifier-layer thickness (Å)[c]	Curve shape[d]	Estimated length of the emulsifier (Å)[e]
Dodecylbenzence sulfate				
0.1	0.47	1.6		15
Fatty acid monoethanolamid ethoxylate (7EO)				
10	0.27	90		54
Fatty acid monoethanolamid ethoxylate (13EO)				
7	0.20	45		75
Fatty acid monoethanolamid ethoxylate (18EO)				
10	0.23	59		93

Adapted from Östberg et al. (1995)

[a]The emulsifier concentration calculated on the oil phase

[b]The radius is shown as $D(3, 2)/2$

[c]The apparent emulsifier layer, estimated assuming that all emulsifier is estimated at the interface

[d]The curve shape shows the dependence for the apparent emulsifier layer of the emulsifier concentration

[e]The estimated length of the emulsifier molecule is estimated from the chemical formula or from measurements in the corresponding lamellar phase

layer. The second component adsorbs to a droplet displaying the characteristic properties of the primary adsorbing emulsifier. This usually means a more hydrophilic surface, which can be expected to reduce the adsorbed amount. However, in some cases, the presence of certain groups increases the adsorption of specific substances. For example, the effects of emulsifiers on protein adsorption is essential in most food applications.

Ethoxylated surfactants usually give a strong reduction of protein adsorption. Courthaudon et al. (1991b) have shown $C_{12}EO_8$ totally displaces all adsorbed β-casein from an emulsion system. Similar effects have also been obtained with emulsions formed with polysorbates (Dickinson and Tanai 1992) and with monoglycerides (Hall and Pethica 1967). On the other hand, egg yolk PC did not reduce the adsorbed amount of β-casein more than about 20% (Courthaudon et al. 1991a).

The adsorption of a range of plasma proteins at various phospholipid surfaces has been characterized using ellipsometry (Malmsten 1995). A large variation of the adsorbed amount was obtained, depending on the combination of protein and phospholipid. Purified PC and PE gave low adsorbed amounts, while phosphatidic acid enhanced the adsorption of fibrinogen by a factor of 5 compared to a bare hydrophobic surface.

6.8 Conclusions

Emulsifiers form the basis for a wide range of essential stability, quality, and efficiency functions in food formulations and remain the preferred choice in most cases because of their simplicity and safety. Complex physicochemical emulsification phenomena occur in formulations and it is important to understand the interactions of emulsifiers and other species using the above information. Complementary reviews on polymeric and steric stabilizers (Heertje 2014) as well as other types of emulsifiers like proteins (Kravlova and Sjöblom 2009) are also recommended.

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Chapter 7
Emulsifiers in Dairy Products and Dairy Substitutes

Stephen R. Euston and H. Douglas Goff

7.1 Introduction

Bovine milk has been an important source of food for human beings for thousands of years. Not only is milk a very nutritious food in its own right, but it is also a very versatile starting point for many other dairy products.

Milk is a complex food emulsion and colloidal sol. Table 7.1 gives the composition of whole cow's milk. The emulsion is composed of fat droplets dispersed in an aqueous phase containing protein. The protein is in the form of both casein micelles, which are themselves colloidal particles, and free in solution as whey protein. A considerable reserve of knowledge has been assembled on the structure and properties of milk proteins (Swaisgood 1992). The fat droplets are stabilized by an adsorbed layer of protein and phospholipid called the "milk fat globule membrane" (MFGM), which is distinct from the aqueous phase protein (Walstra and Jenness 1984). The average composition of the MFGM has been estimated to be about 48% protein, 33% phospholipid, and 11% water, with the remainder made up of other minor lipid components (Walstra and Jenness 1984). The phospholipid fraction of the membrane is composed of lecithin, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositide, plasmalogens and sphingomyelin. Phospholipids are important food emulsifiers in their own right. The contribution that they make to the stability of the milk fat globule is not well understood, but their use as food-grade emulsifiers has been the subject of extensive fundamental research (Courthaudon et al. 1991; Dickinson et al. 1993; Dickinson and Iveson 1993).

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Table 7.1 Approximate composition of bovine milk

Component	Average content (wt%)	Range (wt%)	Average dry matter (%)
Water	87.3	85.5–88.7	
Solids, nonfat	8.8	7.9–10.0	69.0
Fat in dry matter	32.0	21.0–38.0	
Lactose	4.6	3.8–5.3	36.0
Fat	3.9	2.4–5.5	31.0
Protein	3.25	2.3–4.4	26.0
Casein	2.6	1.7–3.5	20.0
Mineral substances	0.65	0.53–0.80	5.1
Organic acids	0.18	0.13–0.22	1.4
Miscellaneous	0.14		1.1

Because of its biological origin, milk is particularly susceptible to microbial and physical deterioration. This severely limits the shelf-life of raw milk. To overcome this milk can either be heat treated to kill bacteria or converted to other products that are more stable due to a decrease in pH, lowered water activity or the presence of salt. Until this century the conversion of milk to butter, cream, ice cream, and various types of cheese had been more of a craft than a science. It is only relatively recent that an albeit incomplete understanding of these processes has been available. It is now understood that the formation of these milk-based products is a consequence of either the destabilization of the dispersed-phase fat droplets (as in butter, ice cream and whipped cream) or of the dispersed aqueous-phase proteins (as in cheese). To control the structure and stability of these products, the manufacturer can add a range of permitted additives that can be either naturally occurring or artificial. One of the most versatile of these additives are the low molecular weight emulsifiers.

In the following pages, the major emulsifier-containing dairy and imitation dairy products will be reviewed. A brief description of their production will be given where relevant, with emphasis on the role that emulsifiers play in the formation and stability of the product.

7.2 Ice Cream

Ice cream and all its related products are characterized by being sweetened, aerated desserts containing milk ingredients that are consumed while being semi-frozen. Usually included in the category are products made from milk fat or non-dairy fat sources; products that span a fat range from low fat (0–2% fat) through the normal range (8–12% fat) to the super-premium products (~16% fat); frozen yogurts; sherbets; and other similar products. Table 7.2 provides a typical range of composition of these frozen dessert products. This section will briefly review the manufacture and ingredients of ice cream, and then will focus on the role of non-protein

Table 7.2 A typical compositional range for the components used in various frozen dessert mix formulations

Component	Premium ice cream	Ice cream	Light ice cream	Lowfat ice cream	Sherbet	Frozen Yoghurt
Fat	14–16	10–14	6–10	2–6	0–2	0–3
Milk solids-not-fat	8–10	10–11	11–12	11–12	2–5	9–12
Sucrose	10–16	10–16	10–16	10–16	20–25	10–16
Corn syrup solids	0–4	4–6	4–6	4–6	0–10	4–6
Stabilizers/ emulsifiers	0–0.3	0–0.5	0–0.5	0–0.5	0–0.5	0–0.5
Total solids	40–45	36–40	32–36	28–32	25–32	28–32
(100 – Water content)						

emulsifiers in establishing ice cream structure, textural quality and shelf life. The reader is referred to three recent texts on ice cream for further details on all aspects of composition and manufacture (Goff and Hartel 2013; Tharp and Young 2013; Clarke 2012) and to the chapter on emulsifiers in the earlier edition of this monograph (Euston 2008) for a review of the earlier literature related to emulsifier functionality.

The manufacturing process for most ice cream and related products is similar (Fig. 7.1). The first step is the preparation of a liquid mix, which includes blending of ingredients, batch or continuous pasteurization, homogenization and ageing. Homogenization forms the oil-in-water emulsion by forcing the hot mix through a small orifice under pressures of 15.5–18.9 MPa (2000–3000 psi gauge), depending on the composition of the mix. Small fat globules (0.5–2 μm) are needed to establish the appropriate degree of fat partial coalescence during subsequent whipping/freezing (Koxholt et al. 2001), although high pressure homogenization can lead to very stable fat globules that are less able to form good structures, due to higher levels of protein adsorption (Hayes et al. 2003; Huppertz et al. 2011; Biasutti et al. 2013). A large increase in the surface area of the fat globules is responsible in part for the formation of the fat globule membrane, comprised of adsorbed materials that lower the interfacial free energy of the fat globules. Emulsifiers play a critical role, as will be discussed below. An ageing time of 4 h or greater at ≤4 °C is recommended following mix processing prior to freezing; ageing allows for hydration of milk proteins and stabilizers (some increase in viscosity occurs during the ageing period), crystallization of the fat globules and membrane rearrangement between emulsifier and protein, as discussed further below. The appropriate ratio of solid:liquid fat must be attained at this stage, a function of temperature and the triglyceride composition of the fat used, as a partially crystalline emulsion is needed for partial coalescence during the whipping/freezing process.

This mix is then whipped and frozen dynamically under high shear in a scraped-surface freezer to a soft, semi-frozen slurry (~−5 °C). Post-freezing processing steps include incorporation of flavouring ingredients to this partially frozen ice

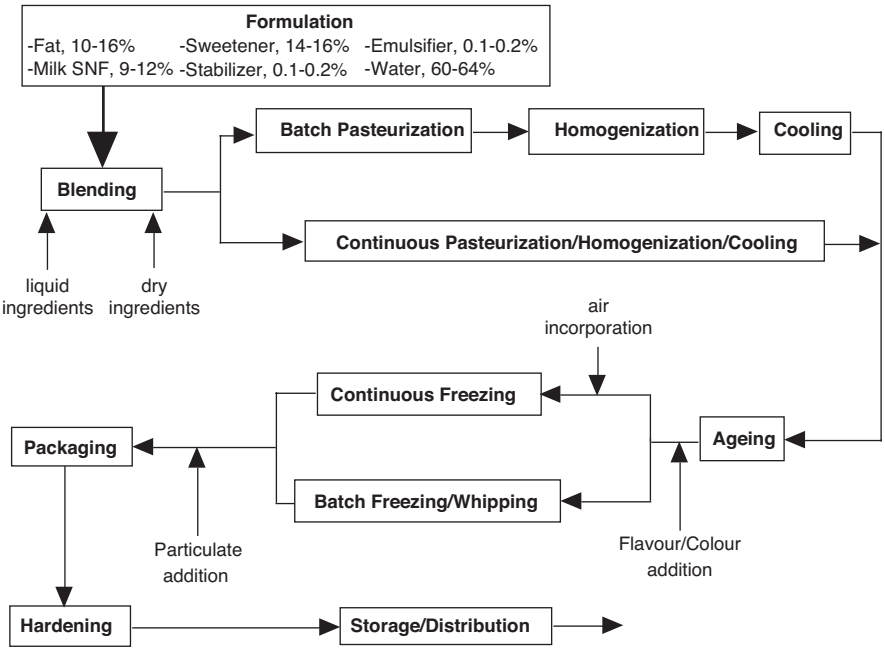

Fig. 7.1 Flow diagram of the ice cream manufacturing process

cream, packaging the product, further freezing (hardening, usually by forced-air convection) of the product, and finally storage and distribution. In the scraped-surface freezer, air is incorporated and whipped into a fine foam (~50% by vol.), water is frozen to ~20–30 μm ice crystals (the threshold of sensory detection is considered as ~50 μm), dependent on the freezing temperature of the mix and the draw temperature of the ice cream from the barrel, and the fat emulsion undergoes partial coalescence to establish a network structure of fat in the product; this will be discussed in more detail below as it relates to emulsifier functionality.

Most ice cream formulations include sources of either dairy or non-dairy fat, milk solids-not-fat, sweeteners, stabilizers, emulsifiers and water (Warren and Hartel 2014). The fat content is an indicator of the perceived quality and/or value of the ice cream, as it produces a characteristic smooth texture in the mouth, helps to give body, and aids in producing desirable melting properties. Milk fat from cream, sweet (unsalted) butter, or anhydrous milk fat is the fat source for dairy ice cream formulations. Vegetable fats can also be used as fat sources in non-dairy ice cream. Blends of oils are often used in ice cream manufacture, selected to take into account physical characteristics, flavour, availability, and cost. Palm kernel oil and coconut oil are most commonly used, often blended with an unsaturated oil to reduce the solid fat content to 50–70% at 0–4 °C (Goh et al. 2006; Bazmi et al. 2007; Sung and Goff 2010). This corresponds approximately to the solid fat content of emulsified milk fat at 0–4 °C (Adleman and Hartel 2001), which is considered as optimum for fat structuring via partial coalescence. The milk solids-not-fat (SNF) contain the

lactose, caseins, whey proteins, and minerals (ash), of the milk or milk products from which they were derived. Both protein concentration and casein:whey protein ratio can vary, depending on ingredients used. Proteins are essential for their functional contributions of emulsification, aeration and water holding capacity/viscosity enhancement, and protein/emulsifier interactions become important in contributing to fat structure development.

Sweeteners provide sweetness but also lower the freezing point and freeze-concentrate as ice is formed, leading to softness and scoopability in the frozen product. In addition to sucrose, corn starch hydrolysis products (corn syrups or glucose solids) are in common use. Ice cream stabilizers are a group of hydrocolloid ingredients (usually polysaccharides) used to produce smoothness in body and texture, reduce the growth of ice crystals and prevent the development of lactose crystals during storage, especially during periods of temperature fluctuation, known as heat shock. Thus, by physical means, they effectively increase the shelf life of ice cream. Stabilizers commonly used include: locust bean (carob) gum, guar gum, and carboxymethyl cellulose, amongst others. Carrageenan is a secondary hydrocolloid used to prevent serum separation in the mix, which is usually promoted by one of the other stabilizers. Small-molecule surfactants (emulsifiers) are usually integrated with the stabilizers in proprietary blends but their function and action is very different from that of the stabilizers, and this will be discussed extensively below. Liquid flavours and colours, those that are to be homogeneous throughout the frozen product, are added to the flavour tank prior to scraped-surface freezing, while particulate flavours are added post-freezing prior to packaging.

The structure of ice cream is quite complex, being both an emulsion and a foam, and the continuous phase is also freeze-concentrated and embedded with ice crystals (Goff 2002). The concomitant whipping and freezing process occurring in the scraped-surface freezer accounts for the formation of the freeze-concentrated unfrozen, continuous phase and all of the discrete phases of ice cream structure: the ice crystals, the air bubbles and the partially-coalesced fat globule structure (Fig. 7.2) (Goff et al. 1999; Muse and Hartel 2004). This structure, in turn, dictates the final texture as consumed. Emulsifiers contribute to the development of the colloidal (fat and air) structure. The milk fat exists in 0.5–2 μm globules that have been formed by the homogenizer and subsequently partially crystallized (50–70% solid fat) by the cooling and ageing process. When the mix is subjected to the whipping action of the scraped-surface freezer, the fat emulsion begins to partially coalesce and the fat globules begin to aggregate. The fat crystals within the droplets protrude through the surface and initiate fat-fat contact between neighbouring droplets, but also then help to retain the shape of the individual droplets, leading to an aggregate of fat rather than simple coalescence (Boode and Walstra 1993; Mendez-Velasco and Goff 2012a; Fredrick et al. 2013a, b). Some liquid fat is required to form liquid oil necks at the globule junctions, thus leading to the 3-dimensional network formation that is typical of partial coalescence (Petrut et al. 2016).

The air bubbles that are being beaten into the mix are stabilized by both the adsorption of proteins and by the adsorption of fat globules and aggregates (Xinyi et al. 2010). The combination of air bubbles partially stabilized by fat

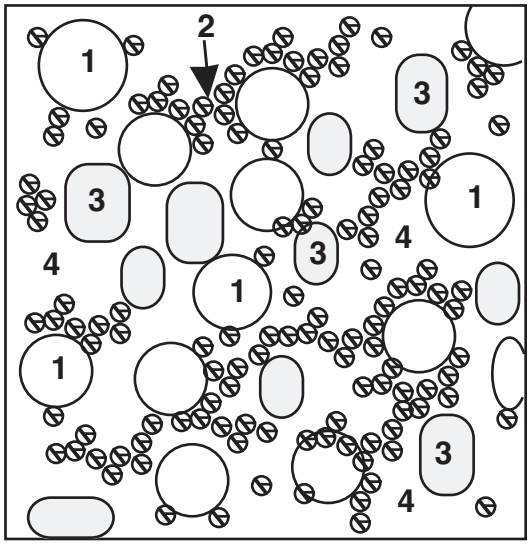

Fig. 7.2 Schematic diagram of the physical structure of ice cream: (1) air cells, (2) fat globules and partially-coalesced fat surrounding air cells, (3) ice crystals, (4) unfrozen, freeze-concentrated solution of dissolved and suspended solids

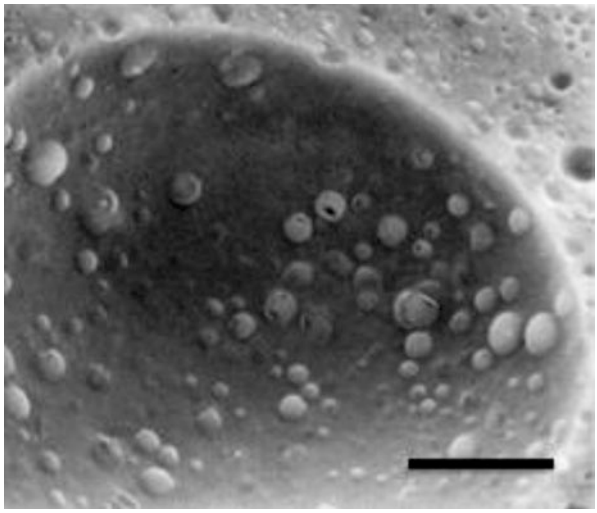

Fig. 7.3 Cryo scanning electron micrograph of a foam bubble in ice-cream. Note the partial coverage of the air bubble surface by fat globules. Fat globules can also be seen in the foam lamellae outside of the droplet (bar = 10 μm)

globules (Fig. 7.3) and partially-coalesced fat aggregates in the bulk phase (Fig. 7.2) results in desirable structural and textural qualities both at the time of draw from the barrel freezer, to allow good shape retention during forming of products, and during consumption, resulting in a smoother, creamier product with slower structural collapse (meltdown). This process is similar to that which occurs during the whipping of heavy cream as the liquid is converted to a semi-solid with desirable stand-up qualities and mouthfeel (Goff 1997), as will be discussed in the next section.

Non-protein emulsifiers such as mono-glycerides or polysorbates have been used in ice cream mix manufacture for many years. They function to:

- promote nucleation of fat during aging, thus reducing aging time (Fredrick et al. 2013a);
- improve the whipping quality of the mix, due to their contribution to the proteinaceous air interface, resulting in reduced air cell sizes and homogeneous distribution of air in the ice cream;
- produce a dry and stiff ice cream as they enhance fat partial coalescence/destabilization, facilitating molding, fancy extrusion, and sandwich manufacture;
- increase resistance to shrinkage and rapid meltdown (Cropper et al. 2013), due to a combination of the above two factors;
- increase resistance to the development of coarse/icy textures, due the effect of fat agglomerates, more numerous air bubbles, and thinner lamellae between adjacent air bubbles on the size and growth of ice crystals (Barfod 2001);
- provide smooth texture in the finished product, due to fat structuring and the interaction of fat agglomerates with the mouth during consumption.

In ice cream mixes, there is sufficient protein present to adequately emulsify the mix, so emulsifiers are not needed for fat emulsification in the classic sense. Their mechanism of action in promoting fat destabilization can be summarized as follows: they lower the fat/water interfacial tension in the mix, resulting in protein displacement from the fat globule surface and more exposure of the crystalline surface, which, in turn, reduces the stability of the fat globule allowing partial coalescence during the whipping and freezing process. In addition to protein displacement, the surface-induced nucleation of fat crystals may also help to create a fat globule surface with sufficient protruding crystals to enhance partial coalescence. Fredrick et al. (2013a) have looked extensively at the effect of emulsifiers on surface nucleation of emulsified fat, as will be discussed subsequently in the next section on whipped cream. The combination of protein displacement and surface rearrangement leads to the formation of a structure of the fat in the frozen product that contributes greatly to texture and meltdown properties (Bolliger et al. 2000). Therefore, both emulsifier type and concentration will affect these properties.

Depending on the type of proteins and the type of emulsifiers, the surface adsorption is approximately 10–12 mg protein per m^2 of fat in the absence of emulsifier and 3–6 mg protein per m^2 of fat in the presence of emulsifier (Barfod et al. 1991; Bolliger et al. 2000). Proteins are large, bulky macromolecules that exist mostly on the aqueous side of the fat interface compared to the very small mono-glycerides, which exist mostly on the fat side of the interface. Thus the thickness of the membrane and hence the stability of the globule to shear is greatly reduced from the action of the emulsifiers. The total quantity of adsorbed protein is not the only determinant factor of partial coalescence when it comes to proteins, however. The traditional ratio of proteins in milk solids is about 80% casein micelles and 20% whey proteins. However, when these ratios are modified by the use of whey protein ingredients in the mix, the integrity of the droplet membrane can be affected. Casein micelles are in the range of 50–200 nm in diameter. When they adsorb to fat droplets, the hydrophobic interior

spreads at the fat interface but the micelle remains as a bulky protrusion into the aqueous phase. The emulsifier can easily penetrate into the area around the micelle to initiate displacement. Generally, whey proteins provide thinner membranes than casein micelles but the membrane is more cohesive and coverage of the surface area more complete compared to casein micelles, which makes displacement of whey proteins by emulsifiers more difficult, and whey protein stabilized droplets less prone to fat destabilization (Segall and Goff 1999, 2002; Daw and Hartel 2015). Relkin et al. (2006) showed that heat aggregation of whey proteins prior to emulsification improved the extent of fat destabilization by making them easier to displace by surfactants. Likewise, sodium caseinate will be harder to displace from adsorbed fat surfaces by small-molecule emulsifiers compared to micellar casein, leading to a more stable emulsion with less partial coalescence upon whipping/freezing (Euston et al. 1995a, b; Davies et al. 2000; Zhang and Goff 2004).

The traditional ice cream emulsifier in old recipes was egg yolk. 1–2% yolk is required to induce sufficient structure similar to the mono-glycerides. However, the use of eggs or egg yolk has been replaced in modern formulations by specific ingredients that deliver much greater functionality at much less cost and lower concentration. Emulsifiers used in ice cream manufacture today are of two main types: the mono- and di-glycerides and the sorbitan esters, or blends of both. Typical concentrations in use are 0.1–0.2% mono- and di-glycerides, perhaps with 0.02–0.04% polysorbate 80. For ice cream applications, mono- and di-glycerides are usually greater than 40% alpha mono-glyceride in content, as it is this form which is most amphiphilic and hence functional. The di-glyceride portion is very fat soluble so contributes much less functionality. Specific glycerol esters, such as glycerol monostearate (or a blend of saturated monoglycerides) or glycerol monooleate (or a blend of unsaturated monoglycerides), can also be used rather than the more random form of mono- and di-glycerides, but they are more expensive to process.

The degree of saturation of the fatty acids present in mono- and di-glycerides also influences the level of fat partial coalescence with unsaturated fatty acids giving greater destabilization (Barfod et al. 1991; Zhang and Goff 2005; Mendez-Velasco and Goff 2012b). High levels of unsaturated monoglycerides have also been shown to form clusters of plate-like fat crystals in ice creams made with palm kernel oil, which are responsible for producing a high level of dryness and shape retention with excellent meltdown resistance (Granger et al. 2003, 2005; Zhang and Goff 2004, 2005; Bazmi et al. 2007; Sung and Goff 2010; Mendez-Velasco and Goff 2011, 2012b). It is postulated from this body of work that the high mobility of the unsaturated emulsifier is responsible for unique interactions with the specific crystal morphologies of the palm kernel oil, a response that was not seen to the same extent with milk fat.

Polysorbate 80, polyoxyethylene sorbitan monooleate, is the most common of the sorbitan esters used for ice cream applications. Polysorbate 80 is a very active destabilizing agent in ice cream and is used in many commercial stabilizer/emulsifier blends. Although it is normally a component of the stabilizer/emulsifier blend, it can be added in pure form directly to the mix flavor tank post-homogenization and will become effective at enhancing product dryness within a few minutes.

In formulations where it is desirable not to include synthetic emulsifiers, a few other more natural options are available. In addition to the egg ingredients discussed above, buttermilk, condensed or dry, when used as the source of MSNF in mixes, also provides enhanced emulsifying properties to the mix, due to the protein-phospholipid complexes found associated with the natural milk fat globule membrane in milk and enhanced in buttermilk during churning. Segall and Goff (2002) introduced processing modifications to enhance fat colloidal interactions in the absence of emulsifier, by limiting protein adsorption at the fat interface during homogenization and introducing further protein to the mix post-homogenization. Mendez-Velasco and Goff (2011) looked at the potential for producing ice cream higher in unsaturated fat by homogenizing two streams separately: the saturated fat globule stream with high level of emulsifier and weak stability produced the required fat destabilization while the unsaturated oil stream, stabilized with protein, remained intact. The potential for natural phospholipids to substitute for monoglyceride ice cream emulsifiers was studied in Italian-style artisanal ice cream (gelato) by Rinaldi et al. (2014). They concluded that soy phospholipids had the most potential in this application. In this regard, the use of buttermilk powder has traditionally been recognized to provide enhanced emulsification in ice cream (enriched in fat globule membrane material), and novel dairy-derived ingredients higher in fat globule membrane material are now becoming available.

In summary, non-protein emulsifiers are important in colloidal structure development in ice cream through their ability to promote adsorption of fat to the air interface and partial coalescence of the fat emulsion, and this results from a displacement of proteins from the fat globule interface and a rearrangement of the fat crystalline interface, thereby reducing emulsion stability. Monoglycerides are particularly good at this function. The fatty acid composition and the concentration need to be optimized based on fat and protein ingredients and concentrations in the mix, on manufacturing conditions and on end-use product characteristics.

7.3 Whipping Cream, Whipped Cream and Whipped Toppings

As a result of the myriad of products that can fall within this category, this section will be restricted primarily to the effect of non-protein emulsifiers on dairy whipped creams, or where research involving non-dairy fats in whipped topping emulsions leads to enhanced knowledge of such systems. For good references related to whipped cream structure, please see van Aken (2001), Palanuwech and Coupland (2003), Hotrum et al. (2004, 2005) and the chapter in the earlier edition of this monograph (Euston 2008) for a review of the earlier literature related to emulsifier functionality.

Whipping cream is an oil-in-water emulsion stabilized by adsorbed milk protein and (where added) low molecular weight emulsifiers, which is designed by formulation and process to be whipped (aeration in the presence of sufficient shear)

to a stable foam structure. Whipped cream is formed from whipping cream when air is incorporated into the emulsion to form a foam and the fat within this foam forms suitable structure (mostly from partial coalescence) to withhold the foam from rapid collapse. The whipping must be done while the cream is cold or has been cold-aged, so a sufficiently high proportion of the fat is crystallized, in order to develop a partially-coalesced structure. Traditionally, whipping cream is non-homogenized dairy cream of 35–40% fat from milk separation. It can be pasteurized, for a shelf life of 2–4 weeks at 4 °C, or UHT sterilized. The latter, meant to induce extended shelf life of 2–4 months at refrigerated or ambient temperature, often necessitates further formulation development, notably the use of non-protein emulsifiers, to maintain its functionality after the effect of the heating process on the milk proteins (Smith et al. 2000a, b). Homogenization will likewise extend the shelf life, but also then necessitates the use of emulsifiers to maintain functionality after the adsorption of proteins to the fat globule surface during the homogenization process. Recombined creams, homogenized from anhydrous milk fat and suitable serum phase containing various sources of milk solids-not-fat (containing proteins lactose and salts, and derived from skim milk powder, buttermilk powder, milk protein concentrate, whey protein concentrate, etc.), and other suitable ingredients (often sweeteners or starch hydrolysates, and stabilizers), can also be prepared to be suitable for whipping. Here again emulsifiers will be required to establish a stable foam structure by inducing fat partial coalescence.

In recent years there has been much interest in non-dairy whipped toppings (the term “topping” being used to differentiate from dairy whipped cream), where the milk fat has been replaced by another mostly-saturated fat source such as palm kernel oil or coconut oil (so that it is partially crystalline at whipping temperatures of 4–10 °C). These may or may not contain milk proteins, but in the absence of milk protein either an amphiphilic protein and/or appropriate emulsifier blends will be required. The emulsions can either be liquid or dry powders designed to be rehydrated, and then further designed to be whipped. In some cases, the emulsions can be pre-whipped and sold to the end-user, such as a bakery, or consumer as a stable foam, perhaps frozen and needing thawing prior to consumption. Another version is the aerosol cans containing emulsion with a pressurized gas phase, meant to be ejected directly onto a dessert and consumed immediately. These can be from either dairy or non-dairy fats, but in this case there is little if any fat structure formed, and the foams can collapse quite quickly.

Before whipping, the cream has to be sufficiently cold-aged to contain a high proportion of fat crystals within the globules. Ihara et al. (2010) studied the effect of whipping temperature on whipped cream properties. There is a balance between higher overrun at higher temperatures and higher stiffness at lower temperatures, but a temperature range of 7.5–12.5 °C was recommended for dairy cream. During whipping, the foam is stabilized initially by adsorbed protein and any added emulsifier. Prolonged whipping of the cream leads to partial coalescence of fat globules and adsorption of fat globules and aggregates at the air/water interface of foam bubbles. This follows the same mechanism as described in the section on ice cream: initiated by intra-globule fat crystals forming fat-fat interactions while inter-globule

fat crystals hold the shape of the globules thus leading to three-dimensional aggregation, held together by necks of liquid oil (van Boekel and Walstra 1981). In addition, the fat globules aggregate in the aqueous phase of the cream to form a continuous, semi-solid gel-like network structure that traps the air bubbles and prevents them from coalescing, and also traps and holds the serum phase to prevent rapid drainage. Figure 7.4 is a cryo-SEM micrograph showing fat globule adsorption at the air/water interface in whipped cream, and the structure of the partially coalesced fat matrix in the foam lamellae. The adsorbed fat globules contribute to the rheological properties of the foam. By influencing drainage in the aqueous lamellae between air bubbles, the partially coalesced, adsorbed fat globules impart a small but finite yield stress on the whipped product. This allows whipped cream to "stand up" under its own weight even at ambient temperature.

The structure of whipped cream resembles that of ice cream in some ways, or to state that another way, the structure of melted ice cream resembles that of whipped cream. Whereas in ice cream the final structure is partially stabilized by fat globule adsorption at the air bubble surface and by fat partial coalescence in the bulk, but also by freezing of the aqueous phase, in whipped cream the higher dispersed-fat phase content (35 wt% compared to about 10 wt% in ice cream) leads to a higher degree of fat aggregation at the air/water interface. This greater fat adsorption leads to formation of a stable foam without the need for freezing. Comparing this to Fig. 7.3, a cryo-SEM of ice cream, it is apparent that the degree of fat globule adsorption is less in ice cream.

Non-homogenized cream separated from milk will whip satisfactorily without the addition of emulsifiers. If the cream is homogenized prior to whipping and the mean fat globule size reduced, emulsifiers have to be added to aid destabilization. The differences in whipping ability of homogenized and non-homogenized cream can be attributed to the differences in interfacial composition of the emulsion droplets in these systems. Non-homogenized cream contains fat globules stabilized

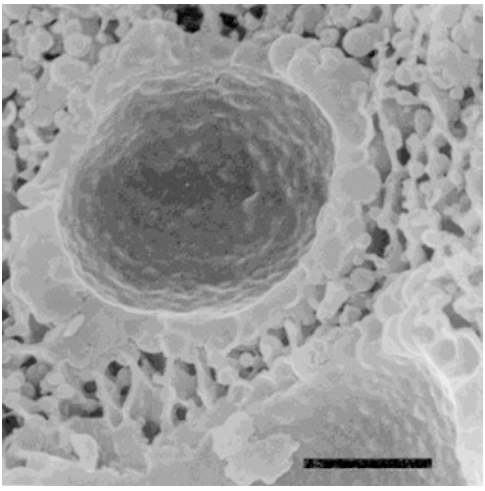

Fig. 7.4 Cryo-scanning electron micrograph of whipped cream. Note the greater coverage of the air bubble surface by fat globules than occurs in ice cream. The partially coalesced fat globule network that gives cream its semi-solid structure is also clearly visible (bar = 20 μm)

by the native milk fat globule membrane (MFGM). The composition of this has been described earlier (Sect. 7.1). After homogenization, the particle size is reduced, interfacial area is increased, and consequently, MFGM on its own is insufficient to stabilize the newly formed interface. Proteins from the aqueous phase (caseins and whey proteins) adsorb to the fat globule and contribute to the interfacial layer. In the early stages of whipping, before fat globule adsorption and partial coalescence occur to any great extent, the air bubbles are stabilized by adsorbed milk serum proteins. Since this air/water interface is composed of the same proteins that surround the fat/water interface in homogenized cream, the difference in interfacial tension between the two interfaces is small. The interfacial tension difference is a driving force for fat globule adsorption at the air/water interface in both cream and ice cream, and if this is low or negligible, fat globule adsorption is reduced. Hence the importance of the small-molecule emulsifiers to such systems: they induce fat-fat interactions and partial coalescence to create fat aggregates that can build stable structure, primarily through their effect on protein displacement from the fat interface as discussed earlier in the context of ice cream (Euston et al. 1995a, b; Davies et al. 2001), and they induce fat adsorption to the air interface by modifying the interfacial properties of both interfaces. In non-homogenized cream the interfacial tension difference between a fat globule stabilized by MFGM and an air bubble stabilized by cream serum proteins are sufficient to act as the driving force for fat globule adsorption at the surface of air bubbles. Of course this is also aided by the thinner membrane of MFGM, the presence of fat crystals at the fat globule surface and by the shearing forces introduced during whipping.

Recombining technologies, homogenizing anhydrous milk fat or other solid fat stock into an aqueous phase, are becoming an increasingly important process for making whipping creams. These encounter the same problems as homogenized cream (i.e., the similarity in composition and interfacial tension between air/water and oil/water interfaces), if they are formulated with milk proteins as the only surface-active material. For this reason, recombined whipping creams, and also homogenized natural creams, contain added low molecular weight emulsifiers. These adsorb at and alter the composition of the fat-droplet surface, thus changing the interfacial tension. Addition of an optimum concentration of emulsifier results in an oil/water interface composed of protein and emulsifier differing sufficiently from the air bubble interface for fat globule adsorption to occur on whipping.

The important role of emulsifiers on promoting fat nucleation and its effect of whipping properties of recombined dairy cream have been demonstrated in recent research by Fredrick et al. (2013a, b). They showed that glycerol monostearate (GMS) formed a two-dimensional crystal at the fat interface upon cooling, which induced interfacial heterogeneous nucleation and accelerated polymorphic change from α to β' crystals. This, in turn, led to smaller, less protruding surface fat crystals. Glycerol monooleate (GMO), on the other hand, had no affect of fat surface nucleation. Interestingly, GMS led to reduced surface protein concentration while GMO did not. GMO, however, reduced whipping time, serum drainage and increased firmness, but GMS led to higher overrun. They thus concluded that the GMO emulsions led to enhanced structure, but not from reduced protein adsorption, rather

from the development of larger surface fat crystals. The protein displacement from GMS, however, likely led to enhanced foamability and higher overrun. A combination of GMS and GMO was therefore suggested to optimize both foamability and foam stability, through divergent effects of the fat droplet surface. By a similar mechanism, tempering of cold-aged cream by warming to 20–30 °C for 30 min followed by cooling and whipping has also been shown to improve partial coalescence by the development of large crystals at the interface (Moens et al. 2016).

Other emulsifiers have also been investigated for their functionality in whipped cream/topping systems. Zhao et al. (2013) investigated the use of sorbitan monostearate in an 18% fat partially hydrogenated palm kernel oil emulsion stabilized with sodium caseinate on its whipping properties, and found an improvement in overrun and a decrease in the formation of large fat aggregates. Sorbitan monostearate, often together with polysorbate monostearate (Polysorbate 60), is commonly used in pre-whipped palm kernel oil/coconut oil emulsion products to give very high foam stiffness and stability by combining the fat soluble and water soluble emulsifiers and their effects on the platelet-like crystals from palm kernel oil, especially after tempering. Munk et al. (2013) examined the effect of lactic acid ester of monoglycerides (LACTEM), in combination with glycerol monooleate (GMO), glycerol monostearate (GMS) or diacetyl tartaric acid ester of monoglycerides (DATEM), on 25% palm kernel oil whippable emulsions sheared in a rheometer. The emulsifiers had minor effect on fat crystallization or polymorphism in bulk fat, however they had a major impact on emulsion stability. The LACTEM/GMO combination led to enhanced partial coalescence and higher viscosity after shear compared to the other emulsifier systems. Kim et al. (2013) also examined the use of LACTEMs in whippable emulsions with 18% interesterified palm fat, compared to saturated or unsaturated monoglycerides. In this system, the LACTEM showed lower emulsion viscosity but higher overrun and higher firmness after whipping compared to the monogylyceride systems. They stressed the differing surface layers and their interfacial shear elastic modulus contributed by these different emulsifiers when interpreting the results, which they suggested should aid in developing better whipping emulsions with good quality and at lower saturated fat content. Polyglycerol monostearic acid ester was added to 45% fat cream or recombined cream by Ihara et al. (2015), and in combination with casein peptide was found to enhance foam stability.

Manufacturer and consumer interest in “clean labeling” has led to enhanced use of more natural sources of emulsifiers. For example, phospholipids are a natural component of milk fat and can be extracted as a by-product when milk fat is concentrated. Due to their phospholipid content buttermilk powders are frequently used as combined emulsifier/protein systems in whipped emulsions. van Lent et al. (2008) prepared recombined dairy cream with milk phospholipid-rich fractions and showed them to be more similar to non-homogenized dairy cream. This was followed up in a study by Phan et al. (2014) who also showed that the addition of fat globule membrane material in a 35% recombined dairy cream led to higher overrun, less serum drainage and reasonable firmness. Borjesson et al. (2015) modified the serum phase of 35% fat unhomogenized cream and found that both a decrease in the

serum protein content and addition of UF permeate or calcium ions led to shorter whipping times, which was ascribed to the effect of calcium on casein at the interface. Similar results had earlier been shown by Zhang et al. (2004), who demonstrated the effect of dissociated caseins on foam interfaces. Allen et al. (2008) also looked at the effect of pH on protein-stabilized whippable emulsions.

Whipped toppings made from a dry powder are an interesting alternative to dairy creams and non-dairy liquid emulsions. These typically contain 30–40% fat from a saturated source such as palm kernel oil or coconut oil, which has been pre-emulsified with a protein source such as sodium caseinate and spray-dried, additional serum-phase protein for foam stability, sweeteners and/or bulking agents such as pregelatinized starch and/or maltodextrin, hydrocolloid stabilizers and emulsifiers. In powdered and rehydrated whipped toppings, similar to dairy whipping creams, the emulsifiers appear to be important in the destabilization of the emulsion, while the protein is important in giving initial stability to the oil-in-water emulsion. The mechanism of emulsion destabilization, however, is different. Whereas whipped dairy and non-dairy creams are stabilized by partially coalesced, relatively intact fat globules adsorbed at the air/water interface, whipped toppings are stabilized by crystalline fat at the air bubble surface. These products were first studied by Krog and coworkers (Barfod and Krog 1987; Buchheim et al. 1985; Krog et al. 1986), and their work defined the relevant structural characteristics. They showed that part of the fat in spray-dried topping powders is in a supercooled state. When these topping powders are reconstituted in water at low temperatures, they show large structural changes that determine whipping characteristics and foam structure. The emulsion becomes unstable due to spontaneous recrystallization of the supercooled fat. The destabilization of the emulsion is probably promoted by the temperature-dependent desorption of protein from the surface, followed by coalescence. This makes crystallization of the supercooled fat more likely, due to the increased probability of nucleation sites. The kinetics of fat crystallization and emulsion destabilization depend on the type of emulsifier used in the formulation. All of the emulsifiers promote fat crystallization when compared to toppings without added emulsifiers. The effect of PGMS and GMO, however, was greater than for GMS, and this led to more stable toppings. These two surfactants also reduced the protein associated with the fat phase, which also contributed to the increase topping stability. The GMS-containing reconstituted topping emulsion was too stable to allow consequent stabilization of incorporated air (whipping).

In summary, the functions of emulsifiers in whipping cream and whipped toppings are essentially the same as for ice cream, i.e.,

1. They aid in enhanced fat nucleation and the formation of fat crystals at the fat-droplet surface. These crystals are essential for fat globule partial coalescence during whipping.
2. They modify the adsorbed layer composition and interfacial tension of the fat droplet, through reduction of adsorbed protein, which is also essential for fat partial coalescence during whipping.
3. They may participate in the initial foam stabilization.

4. As a result of the above, they reduce whipping time, increase overrun, enhance foam stiffness and decrease the rate of collapse; the net manifestation is the creation of a more stable foam with greater performance.

7.4 Cream Liqueurs

Cream liqueurs are dairy emulsions of high added value. The combination of milk protein-stabilized cream emulsion and high alcohol content make cream liqueurs unique among dairy emulsions. Table 7.3 gives a typical composition for cream liqueur. In practice, many commercial formulations also have small amounts of GMS added. The production of cream liqueurs is governed by the relative poorness of the alcoholic aqueous phase as a solvent for proteins and sugars. The presence of ethanol in the emulsion leads to a number of challenges for the manufacturers. Studies on ethanol containing casein-stabilised emulsions have highlighted the difficulties involved. At high ethanol content (30–40 wt%) the aqueous continuous phase polarity is changed to the extent that the steric-stabilizing κ-casein layer at the surface of the casein micelle collapses, stability is reduced and aggregation occurs (Dickinson and Golding 1998). At lower ethanol contents (20 wt%), however, the oil-water interfacial tension is reduced and homogenization leads to smaller oil droplets that have an enhanced stability (Dickinson and Golding 1998). Espinosa and Scanlon (2013) have identified the stable region for ethanol-containing oil-in-water emulsions stabilised by sodium caseinate. They found that ethanol concentrations from 8 to 32 wt% gave stable emulsions. Even in this region, however, emulsion properties were different. Emulsions made with ethanol content in the range 22–32 wt% showed Newtonian rheological behaviour, whilst stable emulsions made with ethanol below 22 wt% exhibited shear-thinning which is characteristic of flocculated systems (Espinosa and Scanlon 2013). Studies of this type will prove very useful for manufacturers in understanding and optimizing cream liqueur product stability.

Two commercial processes are in common use (Banks and Muir 1988), namely, the single-stage process and the two-stage process. Figure 7.5 presents flow charts for both processes. The main difference between the two processes lies in the stage

Table 7.3 Typical composition of a cream liqueur

Component	Composition (wt%)
Milk fat	14
Added sugars	17.5
Sodium caseinate	3.0
Nonfat milk solids	2.3
Alcohol	14
Water	49.2

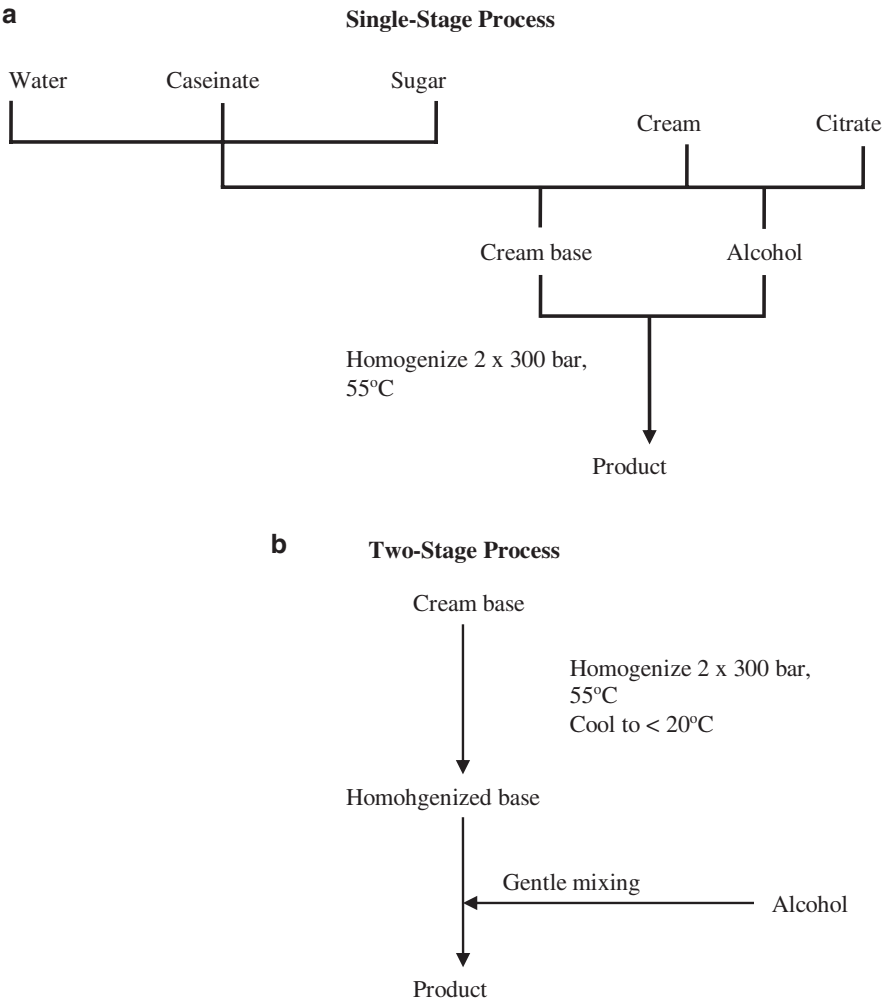

Fig. 7.5 Flow diagrams for the process of manufacture of a cream liqueur in (**a**) a single stage and (**b**) two stages. (From Banks and Muir 1988. Reprinted by permission of Elsevier Applied Science Publishers)

at which the alcohol is added. In the single-stage process this is prior to homogenization, whereas in the two-stage process it is after homogenization. Banks and Muir (1988) found that homogenization in the presence of alcohol leads to the formation of fewer large fat globules, and as such is preferable in terms of emulsion stability. A characteristic of cream liqueur production is the harsh homogenization conditions used (two passes at 300 bar). This results in a product in which more than 97% of the fat droplets have a diameter less than 0.8 μm. Heffernan et al. (2011) have studied the efficiency of different types of homogenizer on the particle size and stability of ethanol containing emulsions. Microfluidizers were found to produce the small-

est and most stable droplets, with other techniques such as an orifice nozzle or a radial diffuser homogeniser producing larger droplets with a wider distribution of sizes (Heffernan et al. 2011).

A second factor favouring formation of smaller droplets is the significant lowering of interfacial tension observed at the oil/water interface when alcohol is added to the aqueous phase (Bullin et al. 1988; Dickinson and Woskett 1988; Burgaud and Dickinson 1990). As a result of the very fine droplet size, the protein in the added cream has to be supplemented by sodium caseinate (to a fat-to-caseinate ratio of 5:1) to provide adequate coverage of the newly formed fat surface by protein (Banks and Wilson 1981). The fine particle size of the dispersed phase fat droplets gives the product an excellent stability with respect to creaming. Banks and Wilson (1981) have noted no signs of creaming in liqueurs with a composition within the range quoted in Table 7.3 after 12 months storage. The high level of added sodium caseinate, however, leads to cream liqueur emulsions being unstable in acid environments. This means that they are not suitable for combination with acid beverage mixers such as lemonade. A cream liqueur that is stable in an acid environment can be made by replacing the sodium caseinate with GMS. The emulsifier replaces milk protein as the primary emulsion stabilizer at the oil/water interface, and the non-adsorbed protein is unable to aggregate the fat droplets when exposed to acidic surrounding (Banks and Muir 1988). Acid stability in this product is gained at the expense of emulsion stability and shelf life. In practice, legal limits in some countries set the concentration of GMS at no more than 0.4 wt%, and so total replacement of caseinate by GMS is not feasible. Many manufacturers add low concentrations of GMS as well as sodium caseinate to cream liqueur formulations. Dickinson et al. (1989a) have shown that, in addition to displacing some, but not all of the milk protein from the fat droplet surface, which presumably infers some acid stability on the product, GMS also improves the stability of a model cream liqueur. When model cream liqueurs were stored at room temperature for 12 weeks, no creaming was observed with added GMS concentrations above 0.5 wt%. Below this level of added GMS a reduced degree of creaming was observed compared to control samples with no emulsifier (Dickinson et al. 1989a). The increased stability was associated with rheological changes in the emulsifier aqueous phase. At low GMS concentrations the emulsions exhibit Newtonian behaviour, whereas above 0.5 wt% a yield stress is found. Dickinson et al. (1989a) postulate the formation of a weak gel-like network in the continuous phase formed by interaction of caseinate with GMS. It is also likely that interaction between caseinate and GMS at the oil/water interface plays a role in the creaming stability. Evidence for interactions between adsorbed caseinate layers and GMS has been reported by Doxastakis and Sherman (1984), who investigated the surface rheological properties of mixed caseinate GMS systems.

An apparent contradiction in the work of Dickinson et al. (1989a) is that although creaming stability is enhanced at GMS level above 0.5 wt%, the shelf life, as tested using an accelerated method at 45 °C, decreases in this region (Fig. 7.6). Dickinson et al. (1989a) point out that whereas weak gels are able to prevent formation of a substantial cream layer, they are also prone to slow syneresis when stored for any length of time. This leads to separation of the aqueous phase and formation of a dis-

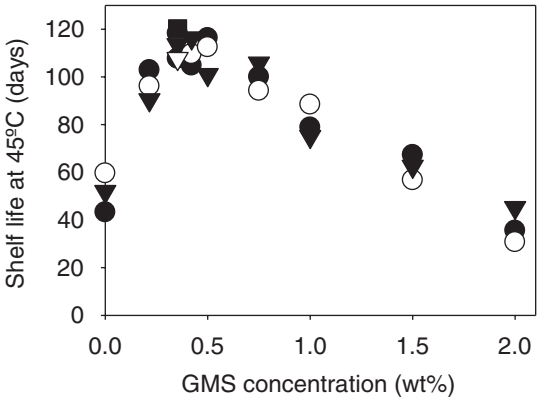

Fig. 7.6 Effect of GMS on the shelf life of simulated cream liqueurs on storage at 45 °C. The time for serum separation to first become visible is plotted against the GMS concentration. Different symbols refer to separate experiments. (Based on Dickinson et al. 1989c, b, c. Reprinted by permission of the Institute of Food Technologists)

tinct, clear serum layer at the bottom of the sample container. Whether this syneresis will occur at room temperature is not certain, and Dickinson et al. (1989a) stress that a correlation between the shelf life at 45 °C and that at room temperature may not follow. Cream liqueurs stored under ambient conditions can have shelf lives of several years. Clearly these are likely to be consumed long before serum separation becomes evident. Since the legal limits on the amount of emulsifier are set at about 0.4 wt%, the problem of gel syneresis is unlikely to be encountered. At a level of 0.4 wt% added GMS, creaming under gravity would not be eliminated completely, but would be reduced to a level acceptable to the consumer (Dickinson et al. 1989a).

7.5 Creams and Coffee Whiteners

Cream products containing 10–20% fat have been popular as coffee creamers for over 50 years (Abrahamson et al. 1988). Coffee creamers and whiteners perform several functions: they give coffee a white colour, reduce bitter taste by complexation of the tannic acids with milk proteins, give the coffee a cream-like flavour, and give body to the coffee (Sims 1989).

Traditionally, coffee cream is produced by simple concentration of milk up to the required fat content. The cream is usually heat treated using a UHT process, homogenized either before or after heating, and packed asceptically to give a long shelf life. Emulsifiers are not usually added to this product. More recently, with the advent of recombining technology and with the preference of some consumers for vegetable oil-based products over those that contain milk fat, new products have appeared that require the addition of emulsifiers if a stable formulation is to be manufactured. If a recombined coffee cream is produced, the formulation is more complex than for natural, concentrated coffee cream (Zadow 1982).

The recombining process involves a two-stage homogenization with an 18 MPa first stage and a 3–4 MPa second stage. Presumably the emulsifiers are added to aid in the homogenization process by reducing the energy required to form the fat/water

interface. It may also be assumed that since in natural coffee creams the milk fat is already in a dispersed state, the energy required to reduce the particle size is less, and so emulsifiers are not needed. However, Zadow (1982) states that emulsifiers and stabilizers are required only if the product is to be given a high heat treatment (a UHT process or steam injection), which suggests a role for emulsifiers in product heat stability. Evidence exists to support this hypothesis and will be dealt with in more detail in Sect. 7.8.

Whereas coffee cream and recombined cream are used in a liquid form, coffee whiteners based on vegetable fat are also popular in dry powder form. Typically, formulations for liquid and dry powder coffee whiteners have differing compositions (Si 1991). Tartaric acid esters of monoglycerides are common emulsifiers in liquid and dry powder coffee whiteners, although these can be replaced with other water-soluble surfactants, such as Tween 60.

Si (1991) states that the function of the emulsifiers in coffee whitener is to improve whitening ability and to aid powder dispersibility in coffee. Knightly (1969) has found that GMS is more effective in improving powder dispersibility, whereas Tween 60 is better at improving the rate of solution of the powder. Optimum whitening ability is attributed to small fat globules and a narrow particle-size range, and its attainment in coffee whitener has been attributed to the presence of GMS and its derivatives (Si 1991). Whitening power in a dispersion is related to the surface area of the dispersed particles. The higher the surface area the greater the light reflectance from the dispersion and thus the greater the whitening effect. This is true for both dairy coffee creams and non-dairy coffee whiteners. Leo and Betscher (1971) have noted that there is an optimum particle size range for optimum optical density and whitening power of the dispersion. Over-homogenization of a coffee whitener formulation is known to result in a loss of whitening power.

The influence of emulsion droplet colloidal properties has been studied in detail by McClements and co-workers from both the theoretical and experimental perspective (Chanamai and McClements 2001; Chantrapornchai et al. 1998, 1999a, b, 2001a, b; McClements 2002a, b; McClements et al. 1998). There results have shown that the whiteness of an emulsion is strongly related to both droplet size and droplet concentration. Emulsion lightness increases up to a maximum as the droplet size increases, and then starts to decrease if the droplet size increases above a critical radius (Chantrapornchai et al. 1998). This confirms the finding of Leo and Betscher (1971) that there is an optimum particle size range for optimum whitening power of a coffee whitener.

The emulsifiers added to powdered formulations prior to spray drying are capable of stabilizing the emulsion in the liquid form. Sodium caseinate is usually required to give stable fat droplets in the dried powder (Sims 1989), since an adsorbed proteinaceous layer is better able to withstand the extreme conditions in the drier. Because sodium caseinate is required at high concentration (typically in the range 3–15%), ways of reducing the amount in coffee whitener have been sought as a cost saving exercise. One way of doing this is to use sodium (or calcium) stearyl lactylate or sodium stearyl fumarate as an emulsifier. Miller and Werstak (1983) have used 2.5% monoglycerides plus sodium-2-stearyl lactylate (SSL) in the approximate ratio 7.3:1.

They claim a reduction of sodium caseinate to 60% of that required in normal formulations. The function of SSL appears to be through its ability to form a complex with sodium caseinate (Leo and Betscher 1971). It is likely that this interaction results in improved fat encapsulation in the dried state through increased interfacial rigidity of the adsorbed layer. This is analogous to the increased emulsion coalescence stability observed when GMS complexes with protein adsorbed at the oil/water-emulsion interface (Doxastakis and Sherman 1984; Rivas and Sherman 1984).

Shelf stability of the emulsion is an important factor to be considered when formulating dairy creams. Dairy creams are susceptible to a phenomenon called rebodying whereby they thicken when the fat in the emulsion droplets is melted and then recrystalised (Boode et al. 1991; Noda and Yamamoto 1994) and appears to be related to changes in fat crystal structure on recrystallization (Boode et al. 1991). Mutoh and co-workers (2007) have studied the inhibitory effect of certain emulsifiers on the solidification of fat in emulsion droplets after heating, and the effect this has on partial coalescence. They tested several emulsifiers and found that two, a low HLB sucrose ester and citric acid esters of monostearate were able to delay the onset of solidification, and hence protect against rebodying due to partial coalescence. For the sucrose ester, the results suggested that the emulsifiers modified the crystallization behaviour of the fat such that smaller crystals were formed which reduced the extent of partial coalescence. For the citric acid ester of monostearate the recrstallization behaviour was not affected, but the anionic nature of the emulsifier probably increased the negative charge on the emulsion droplets and stabilised them to partial coalesecence through an electrostatic repulsion mechanism (Mutoh et al. 2007).

Dairy creams that are used for cooking will also often be subjected to acid conditions in addition to heat. Acidic pH will cause creams to destabilise often through interaction between proteins on the surface of the fat droplets. Ihara et al. (2011) found that acid stability of cream of fat content above 40% could be improved by addition of buttermilk to the cream. This was attributed to the presence of phospholipids in the butter milk. Simply adding phospholipids or lysophospholipids to the cream did not improve acid tolerance. This suggests that the protein in the butter milk also plays a role possibly through protein-phospholipid complex formation, with the adosorption of the complex to the cream emulsion droplet surface contributing to acid stability.

7.6 Cheese, Processed Cheese and Cheese Products

The addition of emulsifiers to traditional cheese has been reported only a few times in the literature (Drake et al. 1994, 1996). In large part this is due to the regulations in many countries that prohibit the use of additives in traditional products. However, food manufacturers are always looking to manufacture new products with novel textures, tastes and functionality, and this has led to the development of cheese-based and dairy analogue products that are not required to adhere to the strict legislation for natural cheese.

One of the first cheese products was processed cheese, the manufacture of which dates back approximately 100 years. Originally it was used as a way of increasing the shelf life of cheese and improving the palatability of lower quality cheese (Caric et al. 1985). To manufacture processed cheese, the cheese raw material (a mixture of rennet and fresh cheeses) is first cleaned, chopped, and heated at 70–82 °C with emulsifying salts and other additives. In this instance, the term emulsifying salt is a little misleading, as they are not low molecular weight (nor proteinaceous) emulsifiers, nor do they play a direct role in creating a fat droplet dispersion. Their main effect in this instance is to increase the solubility of the aggregated cheese proteins through sequestration of calcium, thus improving the emulsifying ability of the caseins. Heating and water addition are often combined by using direct steam injection. The pH of the mix is lowered to 5.6–5.8 using organic acids and the product is then extruded into packages (Rosentahl 1991). Alternatively, the correct pH can be obtained by careful selection of a blend of polyphosphate emulsifying salts, which have some buffering capacity in this application. The final product can have 15–25% fat and up to 58% water.

Processed cheese is a dispersion of fat droplets in a concentrated, gelled protein network. This is in contrast to cheese itself, where the fat is not found as discrete droplets, but forms a semi-continuous phase throughout the protein gel. Emulsion stability in the fat droplets is controlled, primarily, by adsorbed caseins or hydrolyzed casein fractions. Some manufacturers add mono- and diglycerides as emulsifiers. The structure and texture of processed cheese is closely linked with the size and distribution of fat globules in the cheese (Thomas et al. 1980; Shimp 1985). If the fat in a processed cheese is weakly homogenized and large fat droplets are formed, the cheese is soft and melts easily. If the fat droplets are small, the cheese is hard and non-melting.

To control the structure of processed cheese, so-called emulsifying salts such as polyphosphates are added. Although these are not surface-active they play an important role in modifying the emulsifying activity of the surface-active caseins. Caseins bind calcium, and this has the effect of reducing their solubility, and thus their emulsifying ability. Emulsifying salts have a higher affinity for calcium than do the caseins, and thus they are able to improve the solubility and emulsifying properties of the caseins. Emulsifying salts are of two types: those that bind calcium relatively weakly and those that bind calcium more strongly. Weak emulsifying salts have a modest effect on the emulsifying properties of the caseins and lead to the formation of a soft cheese with relatively large fat droplets. Strong emulsifying salts give a greater improvement in the emulsifying capacity and result in a hard cheese with smaller fat droplets.

The use of low molecular weight, surface-active emulsifiers in processed cheese (Tweens and Spans) was first investigated in the 1950s (Holtorff et al. 1951). They are not as good as emulsifying salts at promoting structure formation in processed cheese, and in some cases they act to destabilize the fat emulsion by protein displacement from the surface.

Concern has been expressed over the non-nutritional effect of forming a phosphorous/calcium complex in processed cheese, as the calcium is less easily adsorbed in this form. The supplementation of emulsifying salts by monoglycerides has been

investigated as a way of reducing the concentration of emulsifying salts. Gavrilova (1976) produced processed cheese of improved rheology and shelf life using an emulsifying salt/monoglyceride mixture. Zakharova et al. (1979a, b) achieved a 50% reduction in the concentration of emulsifying salts required by adding 1% monoglyceride to the cheese. The processed cheese produced was reported to be of good quality and to have improved hydrophilic (water binding) properties.

Lee et al. (1996) have studied the effect of adding small concentrations of low molecular weight surfactants as co-emulsifiers in combination with emulsifying salts in a model processed cheese. The surfactants used were sodium dodecyl sulphate (SDS, an anionic surfactants), cetyl-trimethyl ammonium bromide (CTAB, a cationic surfactant), lecithin (a zwitterionic surfactant), and glycerol monostearate (GMS, a nonionic lipophilic surfactant). Although the addition of surfactant was observed to result in a reduction in fat-droplet size, the degree of uniformity of the dispersion differed between emulsifiers. In contrast to previous reports that smaller more evenly dispersed fat droplets gave firmer cheeses (Thomas et al. 1980; Shimp 1985). Lee et al. (1996) found no relationship between processed cheese hardness and emulsion structure in the presence of emulsifiers. They concluded that electrostatic interactions between the emulsifier and the protein played the major role in determining the rheological properties of the cheese. The anionic surfactant SDS gave the softest cheese, the cationic surfactant CTAB the hardest. GMS and lecithin gave cheeses with rheological properties little different from the control with no added emulsifier.

Vial et al. (2005a) have designed a formulation for a light-textured foamed fresh acid cheese product that has improved spoonability, spreadability and a more homogeneous texture than conventional fresh acid cheeses. The final product contained 15% air by volume, which contributed to the altered properties of the product. The structure, texture and properties were found to be sensitive to the level of addition and type of emulsifier added (Vial et al. 2005b). Mono di-glycerides were found to reduce the ease of foaming in the formulation, whilst phospholipids in combination with whey protein concentrate gave softer textures. Low molecular weight emulsifiers had little impact on the stability of the product, with this being improved by the addition of WPC.

For traditional cheese products much of the research on emulsifier incorporation into the cheese structure has concentrated on improving the texture of reduced fat cheese (Drake et al. 1994, 1996). One of the nutritional criticisms of traditional cow's milk cheese is that it contains relatively large amounts of saturated fat. Thus, much effort has been put into either reducing the fat content of cheese or incorporating "healthier" polyunsaturated fats into the cheese matrix. Early attempts at reduced fat cheese often led to products that had a poor texture, flavour and melting properties (Lobato-Calleros et al. 2001; Tunick et al. 1999). Swanson and co workers (Drake et al. 1994, 1996, 1999) have found that emulsifiers can act in a similar way to fat-replacers by either improving water binding in the protein matrix, or by promoting the formation of mixed emulsifier-protein aggregates of a similar size to fat globules. These aggregates mimic the effect of fat in the cheese matrix and improve the texture properties of the cheese.

Other studies have looked at the effect of emulsifier blends on the properties of cheese containing canola oil as a functional food ingredient (Lobato-Calleros et al. 2003). One of the problems with fat replacement in cheese is that saturated fats are solid at storage (and eating) temperatures, whilst polyunsaturated fats are liquid. This has texture implications for the cheese, if it is desirable to mimic the texture of the saturated fat containing cheese. This has led to the investigation of the use of emulsifier blends to control the size of the polyunsaturated fat droplets in an attempt to modify the rheological properties of the product ingredient (Lobato-Calleros et al. 2003). The rationale for this approach is the knowledge that low molecular weight emulsifiers are known to alter the textural properties of protein gels and emulsion gels by changing the way in which the proteins interact with themselves and the way fat globules interact with the protein gel matrix.

Studies on, in particular, whey protein denaturation and gelation give some indication as to the mechanisms by which low-molecular emulsifiers affect the properties of protein gels. The situation is complex, with emulsifiers having differing effects depending on the emulsifier types and conditions of gelation. Lipids in whey protein concentrate (WPC) (derived from the original milk source) inhibit the gelation properties of the proteins by competing for hydrophobic binding sites in the protein (Mangino 1992; Morr 1992; Morr and Ha 1993). In contrast, lecithin can enhance gelation or have no effect depending on the conditions (Ikeda and Foegeding 1999a, b). The reason for this is the ability of whey proteins to form differing gel structures depending on the pH and salt concentration (Langton and Hermansson 1992; Botcher and Foegeding 1994; Bowland and Foegeding 1995; Bowland et al. 1995). At low ionic strength, low pH well away from the isoelectric point aggregation occurs in a linear fashion to form a fine-stranded gel (Langton and Hermansson 1992). At high ionic strength and/or pH close to a particulate type gels are formed, and under intermediate conditions, between the two cases above, mixed gel structures form (Botcher and Foegeding 1994; Bowland and Foegeding 1995; Bowland et al. 1995). Foegeding & co-workers (Ikeda and Foegeding 1999a, b; Ikeda et al. 2000) have shown that lecithin increases the gelation rate and gel strength for fine-stranded and mixed gels, but has no effect on particle gels. They hypothesise (Ikeda and Foegeding 1999a, b) that protein aggregation is facilitated by lecithin-protein interactions at low NaCl concentrations, because lecithin is isoelectric over a wide pH range. Consequently, there will be no electrostatic repulsion with the protein. As NaCl concentration is increased, charge screening will reduce the electrostatic repulsive barrier to protein aggregation, and the effect that lecithin has on promoting protein-protein interaction is reduced.

Emulsifiers can have a further effect on the mechanical properties of protein gels when fat droplets are present dispersed throughout the gel network. These so-called emulsion gels are formed when an oil-in-water emulsion is converted to a gel through the action of heat. A gel can be formed at lower protein concentrations than for a solution of the protein, since the fat droplets act as filler particles and increase the effective concentration of the proteins. The effect of lecithin on the mechanical properties of whey protein emulsion gels has been studied by Dickinson et al. (Dickinson and Yamamoto 1996a, b; Dickinson et al. 1996). When lecithin is added

before homogenisation (Dickinson et al. 1996) protein is displaced from the emulsion droplet surface. The droplets are not able to interact with the protein gel network through protein-protein interactions between the adsorbed and bulk phase protein, and they behave as inert filler particles that weaken the gel structure (Jost et al. 1989). Adding lecithin after homogenisation does not cause protein displacement. Under these conditions an increase in the mechanical strength of the gel is seen (Dickinson and Yamamoto 1996a, b), because the adsorbed proteins interact with the gel network, and strengthen it. Dickinson and Yamamoto (1996a) propose that lecithin is able to interact with adsorbed and non-adsorbed protein, thereby enhancing protein-protein cross-links and strengthening the gel.

Emulsifiers other than lecithin also have an effect on whey protein gel strength. The addition of glycerol monooleate (GMO) to WPC emulsion gels leads to a decreased elastic modulus at low additions of GMO, but this is recovered for higher levels of addition (Chen and Dickinson 1999). Again it is believed that the decreased modulus results from protein displacement from the fat droplet surface, which does not allow them to participate in the gel network. At higher levels of GMO addition, the emulsifier aids the formation of a finer initial emulsion (smaller droplets), and when the emulsion gel is formed, even though the droplets do not crosslink with the gel structure, because they are smaller they perturb the protein gel network less (Chen and Dickinson 1999).

The non-ionic emulsifier Tween-20 (T20) has a complex effect on emulsion gel strength (Dickinson and Hong 1995; Dickinson et al. 1996), which is most likely due to its ability to bind with the major whey protein β-lactoglobulin to form a 1:1 complex. If the protein and T20 are present at a molar ratio (R) of protein:T20 of 1:1 ($R = 1$) there is a big increase in the gel elastic modulus for the heat set emulsion gel compared to gels made at lower R. As R is increased to 2, the modulus decreases sharply, and if R is increased above 4 the modulus increases again. The increase in modulus at $R = 1$ is attributed to the 1:1 complex formed between β-lac and T20 (Dickinson et al. 1996). As R is increased there is a displacement of protein displaced from the emulsion droplet surface which amounts to about 90% displacement at $R \approx 2$. This reduces extent of interaction of the emulsion droplets with the aggregated protein network and reduces the elastic modulus. When R exceeds 4 it is thought that mixed micelles of protein and T20 form, and these are able to reinforce the gel (Dickinson et al. 1996).

7.7 Recombined, Evaporated and Concentrated Milks, and Dairy Protein-Based Emulsions

Recombined and concentrated milk products are produced for economic reasons. The cost effectiveness of transporting milk products that have been concentrated by removal of a proportion of the water phase, and the associated increases in shelf life, make milk concentration a viable process. Similarly, it is cost effective to transport dehydrated ingredients for recombination into milk. The function of emulsifiers is,

primarily, to aid in the formation and stabilization of the emulsions. A secondary function, which is claimed by many manufacturers of emulsifiers, is the effect that emulsifiers have on the heat stability of milks and milk products. This is of particular importance in enteral and parenteral emulsion products. These products are either tube-fed to seriously ill hospital patients (enteral formulations) or to new-born or young babies (parenteral formulations). In both cases microbial sterility of the product is very important, as it is undesirable to expose either of these two populations to high levels of bacteria since their immune systems may be suppressed or underdeveloped. As a consequence these types of emulsion are subjected to very intense heat treatments, and this can cause problems with emulsion fat droplet stability.

The effect of emulsifiers on milk and protein emulsion heat stability may be due to two effects. Low molecular weight emulsifiers are well known to compete for interfacial area with proteins and to displace them from the fat droplet surface (Dickinson and McClements 1995). Displacement of protein depends on a number of factors such as the type (oil soluble or water soluble) and concentration of emulsifier (Dickinson et al. 1993; Euston et al. 1995a, b) and environmental conditions such as the temperature (Dickinson and Tanai 1992). The second effect of emulsifiers is that they are capable of binding to proteins and affect their heat stability and their adsorption at surfaces (Bos et al. 1997). The milk whey protein β-lactoglobulin is particularly susceptible to emulsifier-induced changes in heat stability since it has a hydrophobic cleft capable of binding amphiphilic and hydrophobic ligands (Hambling et al. 1992). Puyol et al. (1998) have reported that palmitic acid binding to β-lactoglobulin increases the temperature at which the protein denatures and gels, and Creamer (1995) has shown that binding of SDS or palmitic acid to β-lactoglobulin stabilizes it against denaturation in urea solutions.

In addition to reviewing the effect of emulsifiers on the properties of traditional milk emulsions, we will also summarize the relevant results on the heat stability of simple oil-in-water emulsions stabilized by dairy proteins. These studies have a direct relevance to the heat stability of commercial milks.

7.7.1 Recombined Milk

Recombination of dairy ingredients into milk products is a popular and viable alternative to the export/import of fresh dairy products. It is particularly important in countries where, for various reasons (e.g., transport delays, high temperatures), the shelf life of fresh products prohibits their importation or local production. In such cases, dried dairy ingredients are recombined close to the point of sale, so as to reduce these problems.

Two approaches to recombining of whole milk can be used;

1. Recombination of anhydrous milk fat (AMF), skim milk powder (SMP) and water.
2. Reconstitution of whole milk powder (WMP) with water.

In the past the latter process was, generally, less popular because of problems with the oxidative stability of the fat in the powder during storage. Advances in gas packing of powders, more regular shipping, and use of cooler storage facilities have removed this obstacle. Zadow (1982) noted that the choice of whether to recombine or reconstitute WMP depends on the export strategy of a particular manufacturer. The manufacture of different dairy products is often linked for practical reasons. If a particular manufacturer is making large quantities of butter, this requires separation of the cream (fat droplet) phase from the whole milk. This leaves a skimmed milk stream that is often dried to powdered SMP. Similarly, if cheese is the major product (which is made from whole milk), WMP is usually the major dried form of milk manufactured by the processor. Thus, it makes economic sense for a butter-led industry to have a recombining strategy, whilst a cheese-led industry will have a reconstitution strategy. During the 1970's, an increase in the production of reconstituted WMP was seen. This corresponded to a change from a butter/SMP-oriented export industry to a cheese/WMP-oriented export strategy in countries such as New Zealand and Australia (Zadow 1982).

In the recombination process, AMF, SMP and water are recombined to give a product with the same fat and protein content as whole milk (Kieseker 1983). The skim milk powder is dissolved in the water at 40–55 °C. The fat is added in a molten state, and the mixture is homogenized 14.0–17.5 MPa for the first stage and at 3.5 MPa at 55–60 °C in the second stage. The milk is then subjected to one of three heat treatments: pasteurization at 72.2 °C for 15 s; UHT processing at 135–150 °C for 2–5 s; or in-can sterilization (e.g. 120 °C for 20 min). UHT processing can be by either direct steam injection or indirect heating in a plate or tubular heat exchanger.

Many manufacturers add low molecular weight emulsifiers to the formulation, particularly mono- and diglycerides (Zadow 1982; Kieseker 1983; Sjollema 1987). Emulsifiers in the form of phospholipids can also be added through the practice of replacing up to 20% of the SMP with buttermilk powder (BMP) (Zadow 1982; Kieseker 1983; Sjollema 1987) to give an improved taste.

It is claimed that emulsifiers aid in the formation of the milk fat emulsion during homogenization. Recent research by Mayhill and Newstead (1992), however, suggests that little benefit in terms of emulsion formation and stability is gained by their addition. In the case of mono−/diglyceride emulsifiers, it appears that tradition dictates their presence in the formulation. It is possible that any reduction in creaming due to reduced fat-droplet size in the presence of emulsifier is cancelled out by reduced emulsion stability caused by protein displacement.

7.7.2 Evaporated and Concentrated Milks

Evaporated and concentrated milks are made by removal of water from natural or recombined milks. The technology used to make these products includes evaporation under reduced pressure, reverse osmosis, ultrafiltration, and freeze concentration (Knipschildt and Andersen 1994; Varnan and Sutherland 1994). These concentrated milk products are more susceptible to heat coagulation when UHT processed or sterilized, than are normal concentration milks.

It has been known for some time that the heat stability of skim milk can be altered by surfactant molecules (Singh and Creamer 1992). Anionic surfactants such as SDS have been shown to shift the maximum in the heat stability/pH profile of skim milk to more acidic values and to give a marked increase in maximum heat stability (Fox and Hearn 1978). Cationic surfactants such as CTAB move the maximum heat stability to more alkaline values and give only a slight improvement in the heat stability the maximum (Pearce 1978; Shalabi and Fox 1982). The mechanism by which these changes occur is not known for certain. It has been suggested that binding of the surfactant to casein micelles alters the surface charge, which leads to changes in heat stability (Fox and Hearn 1978; Pearce 1978; Shalabi and Fox 1982). This view is supported by the fact that nonionic surfactants such as Triton X and Tween 80 have no effect on the heat stability of skim milk (Fox and Hearn 1978). Research into the effect of addition of SDS and CTAB on the heat stability of milk proteins is useful only in helping to understand the process of heat coagulation. These surfactants cannot be added to milk products. In addition to this, in most concentrated milk products and in whole milk, the fat globules play a role in heat stability. Surfactants would interact with both the fat-droplet surface and the milk proteins. This makes the process of heat coagulation in fat-containing milks more complicated than in skim milk.

The milk fat globule membrane is known to play a role in the heat stability of milk. In non-homogenized whole milk the fat globules have little effect on heat stability (Singh and Creamer 1992). However, after homogenization the heat coagulation time decreases with increasing homogenization pressure (Singh and Creamer 1992). Obviously, this is an important observation since homogenization of milk is often essential so as to give adequate creaming stability.

It has been known for some time that lecithin can be used to increase the heat stability of homogenized and concentrated milks (Maxcy and Sommer 1954; Leviton and Pallansch 1962; Hardy et al. 1985; Singh and Tokley 1990; Singh et al. 1992). The mechanism of lecithin action has as yet not been elucidated. Lecithin is known to displace protein from the fat-droplet surface (Courthaudon et al. 1991; Dickinson et al. 1993; Dickinson and Iveson 1993) and to complex with milk proteins (Barratt and Rayner 1972; Korver and Meder 1974; Hanssens and van Cauwelaert 1978). Hardy et al. (1985) and McCrae and Muir (1992) also believe that lecithin/protein interactions play a role in heat stability of concentrated milks. Singh et al. (1992) have put forward the view that lecithin may promote the formation of a complex between κ-casein in the micelles and β-lactoglobulin. The formation of the same complex can be promoted by preheating concentrated milks prior to the main heat treatment. This has been shown by Newstead et al. (1977) to have a stabilising effect on the heat stability of recombined evaporated milk. It is interesting to note that, despite evidence of lecithin/protein interactions, Singh et al. (1992) have shown that the heat stability of skim milk is unaffected by lecithin addition. This is powerful evidence for the main stabilizing effect being fat-droplet based.

Milk fat globule membrane (MFGM) material is a natural source of phospholipids (Rombaut et al. 2007) that has been investigated in recombined and evaporated milk products for its ability to improve heat stability. MFGM is found in butter milk

powder (BMP) produced as a by-product of the butter making process. During churning of cream to butter the MFGM is disrupted and separates from the fat with the liquid butter milk. This can be dried to BMP and used as a functional ingredient. As well as MFGM proteins, the BMP also contains phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine and phosphatidylinositol from the lipid membrane surrounding the milk fat globule Patton and Keenan (1975). These phospholipids make BMP a good emulsifying agent (Wong and Kitts 2003).

Kasinos et al. (2014a) have studied the effect of BMP and cream residue powder (CRP), a by-product of milk fat production from cream, on the heat stability of sterilised recombined concentrated milk. The elevated levels of phospholipids in both products were able to protect against heat-induced viscosity and particle size changes when added at levels up to 6%. The protective effect of both powders was proportional to their concentration, and at 6% addition the viscosity and particle size of both BMP and CRP supplemented emulsions was the same as for the starting emulsion without heat treatment. Further studies indicated that the phospholipids acted to displace protein from the surface of the milk emulsion droplets, thus reducing the protein-mediated heat-induced interaction of droplets during processing. Again, this evidence for the effect of phospholipids being fat droplet based.

The same research group have also investigated the use of phospholipids from other sources on the heat stability of recombined concentrated milk (Kasinos et al. 2014c). They demonstrated that sunflower lecithin addition was also able to improve heat stability, and that by hydrolysis of the lecithin to lyso-phospholipid using phospholipase A, it was possible to identify an optimum degree of hydrolysis that gave optimum heat stability (Kasinos et al. 2014c). In a parallel study Kasinos et al. (2014b) also demonstrated that the composition of the phospholipid was important, with longer chain fatty acids inferring greater protection against heat-induced gelation.

7.7.3 Dairy Protein-Based Emulsions

Dairy emulsions are formed by homogenizing fat or oil in the presence of an emulsifying agent, usually a protein. In dairy systems the common protein emulsifiers are the milk caseins and the whey proteins. Of these the caseins are very heat stable (Cruijsen et al. 1994) and casein stabilized emulsions must be heated at high temperature for long times before they become unstable. The whey proteins, on the other hand are globular proteins, and as such they will denature and aggregate on heating. The fact that milk whey protein denaturation only occurs above the temperature range 70–80 °C only exacerbates the problem since common processing temperatures are in this range or above. The obvious response to this would be to remove whey proteins from milk protein based emulsions and to use only the caseins. However, whey proteins are added for their nutritional value, especially in infant formulations based on cow's milk which are designed to have a composition that mimics human breast milk, and also because they are stable at acid pH whereas

caseins are not. In paediatric formulae, milk protein based formulations are made with an increased level of whey protein compared to normal milk (Emmett and Rogers 1997). The reason for this is that human milk has a higher whey protein:casein ratio (60:40) than cow's milk (Emmett and Rogers 1997). The sterility of these infant formulae is of critical importance since they are fed to premature or newborn infants who may not have an immune system that is resistant to common bacterial contaminants. As a consequence these are usually given an intense heat-treatment, such as in-container sterilization (e.g. 120 °C for 20 min) which can lead to instability of the emulsion. To avoid this, the milk proteins in these formulations are usually hydrolysed, i.e. they have undergone enzymatic hydrolysis to break up their native structure and release peptides and amino acids. Hydrolysis can be beneficial for two reasons. The whey protein β-lactoglobulin has been linked with allergy to cow's milk (Cordle 1994; Tormo et al. 1998), and its hydrolysis can remove this by producing small peptide fragments and/or free amino acids, that are more easily digested and absorbed in the gut (Frøkjaer 1994). Secondly, hydrolysis of whey proteins can reduce its susceptibility to heat denaturation. Unfortunately, this does not necessarily mean that dairy emulsions made with whey hydrolysed proteins are more heat stable. Hydrolysed protein form emulsions that are less stable to coalescence, and this is accelerated by heating (Euston and Finnigan 2001). As a consequence, research has focused on how other ingredients affect the heat stability of food emulsions (Euston et al. 2001, 2002). In particular, low molecular weight emulsifiers have been shown to either increase or a decrease the aggregation rate in heated whey protein emulsions depending on the surfactant type (oil or water-soluble) and the concentration (Euston et al. 2001). This was explained in terms of either the ability of surfactants to displace protein from the droplet surface, or their ability to bind to whey proteins (particularly β-lactoglobulin, β-lac) and thus to influence denaturation and aggregation (Euston et al. 2001). McSweeney et al. (2008) compared the effect of monoglycerides and lecithin on the heat stability of model infant formula. Monoglyceride containing emulsions gave a smaller particle size on heating than those containing lecithin. Lecithin, on the hand, increased heat stability whilst monoglycerides decreased it. This increase in heat stability was greater with higher lecithin concentration.

7.8 Other Dairy Applications of Emulsifiers

Emulsifiers have been added to other dairy products to exploit functional properties not normally associated with such emulsifiers. In recombined butter, phospholipids are added as anti-spitting agents, to prevent fat spitting during heating, and monoglycerides have been claimed to provide better "stand-up" properties during storage (Kieseker 1983).

Both sucrose esters and glycerol esters of fatty acids (monoglycerides) are finding a wide range of novel uses. In addition to being good emulsifiers for use in ice

cream (Buck et al. 1986), they are known to improve the mouthfeel in yoghurt (Farooq and Haque 1992), inhibit microbial growth (Conley and Kabara 1973; Kato and Shibasaki 1975; Shibasaki 1979; Beuchat 1980; Kabara 1983; Tsuchido et al. 1987), enhance the thermal death rate of bacteria and bacterial spores (Tsuchido et al. 1981, 1983), and increase the heat stability of bovine serum albumin (Makino and Moriyama 1991). It appears that these functions are a result of their ability to bind to proteins (Clark et al. 1992; Fontecha and Swaisgood 1994).

Monoglycerides in the α-gel phase have been added to low fat yoghurt to mimic the effect of the removed fat (Aguirre-Mandujano et al. 2009). Varying the ratio of monostearin to monopalmitin in the mixture gave good control over texture. Higher levels of monostearin (65 and 87%) gave more viscoelastic gels than the full-fat or low-fat control yoghurts, and the 65% mononstearin sample gave reduced syneresis compared to the controls. These results suggested a composite protein-monoglyceride gel phase was formed that gave a synergistic increase in gel strength.

7.9 Summary

Emulsifiers are very versatile food additives that can be used as aids to emulsion formation (e.g. in coffee whiteners/creamers and recombined products), or in contrast, as emulsion destabilizers in ice cream, whipping cream and whipped toppings. These two functions rely on the classical ability of emulsifiers to act as surface-active agents. In this way they can influence the formation and stabilization of the fat-droplet adsorbed layer and the composition of this layer. This ability of emulsifiers to displace protein from the droplet surface also, probably, accounts for the increase in heat stability of concentrated milks when phospholipids are added.

In a similar vein, displacement of adsorbed caseinate by GMS in cream liqueurs can be used to give increased acid stability to these products. A secondary function of the GMS in cream liqueurs is its ability to interact with proteins, thereby forming a weak gel in the aqueous phase. The associated increase in viscosity gives increased creaming stability. The ability of the emulsifier SSL to interact with caseinate is also exploited in coffee whiteners. The replacement of sodium caseinate in coffee whitener is achieved using SSL. It has been hypothesized (Leo and Betscher 1971) that this is possible because of the increased mechanical strength of a protein/SSL adsorbed layer caused by emulsifier/protein interactions.

In processed cheese and cheese substitutes, the ability of charged emulsifiers to interact with proteins in the cheese matrix may prove a useful way of controlling cheese texture. This would introduce a way of reducing emulsifying salts such as mono- and polyphosphates. The final, but very important, function of some emulsifiers is their ability to act as initiators of fat crystallization. This is a particularly important function in whipped products, and in combination with protein displacement forms the basis of the formation of the whipped foam structure.

A wide range of emulsifiers allowed for food use can be added to achieve the above effects. Of late, consumer opinion has been focused on the "unnatural" nature of synthetic emulsifiers. There is a slow push toward the replacement of synthetic emulsifiers with natural emulsifiers such as milk and soy phospholipid, and milk fat-derived mono- and diglycerides. The future may see a large increase in products such as BMP, which is rich in natural milk phospholipid as well as protein, and milk fat that has been enriched in mono- and diglycerides by processes such as controlled glycerolysis of triglycerides.

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Chapter 8
Emulsifiers in Infant Nutritional Products

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8.1 Introduction

Infant formulae are specially formulated milks for babies and young children. These important nutritional products are available in several forms including convenient ready-to-feed liquid products, concentrated liquid products and powders that are reconstituted for consumption. The formation and stabilisation of an oil-in-water (o/w) emulsion is an integral step in the manufacture of all of these products; this is generally achieved by homogenising the oil phase, usually a blend of vegetable oils such as palm, coconut, soybean and sunflower oils, in an aqueous phase consisting mainly of carbohydrate, proteins, minerals and vitamins. The proteins together with low molecular weight food grade emulsifiers form a membrane that stabilises the oil droplets against coalescence.

This review includes some background information on the various types of nutritional products and describes the role of emulsifiers in these products and provides some information on the various production processes, with emphasis on emulsion formation and stabilisation. The typical protein sources and low molecular weight emulsifiers available for use in these products are considered in the context of the regulatory guidelines and restrictions. Finally, the functionality of emulsifiers, both protein and non-protein types, in the formation and stabilization of emulsions will be discussed.

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8.2 Types of Infant Formulae

A first age-infant formula is intended for consumption by infants from birth to ~4–6 months of age. The majority of formulae in this category can be considered as reformulated bovine milk, which has been modified to reflect the energy content and nutrient profile of human milk. Diluting the protein content, replacing the milk fat with vegetable oils and altering the mineral and vitamin profile of bovine milk are important features of this reformulation. In addition, the whey protein: casein (W:C) ratio may be adapted to reflect the ratio in human milk. This is achieved by enriching the formula with whey proteins, thus converting the W:C ratio from that of bovine milk, which is approximately 20:80, to that of human milk, which is approximately 60:40.

In the cases of infants for whom the standard milk protein based first age formula is not suitable, other options are available. Formulae based on isolated soy protein are available for infants who display intolerance to milk protein. Where the carbohydrate source is other than lactose, soy protein based formulae may also be suitable for infants who display lactose intolerance. Lactose-free milk protein based formulae are available for infants who are lactose-intolerant but who can tolerate milk protein. Formulae based on hydrolysed proteins are available for infants who display milk protein allergy or intolerance. These formulae are classified according to the degree of protein hydrolysis as 'extensively' or 'partially' hydrolysed protein products. Hypoallergenic formulae containing extensively-hydrolysed proteins are generally recommended for atopic infants who have a hereditary predisposition toward developing certain hypersensitivity reactions upon exposure to specific antigens. These formulae are typically bitter due to the exposure of hydrophobic amino acids. More palatable formulae, based on partial protein hydrolysates (90% peptides <6 kDa), have been shown to delay or prevent the onset of allergies in sensitive infants. Only pure amino acid mixtures are considered non-allergenic and elemental diets containing free amino acids are prescribed for infants with highly allergic conditions. Some infants, classed as 'small for gestational age' have increased nutritional requirements and high-caloric, or nutrient dense formulae may be prescribed. Infants that are prone to gastro-oesophageal reflux (GOR), i.e. the involuntary passage of gastric contents into the oesophagus, may be fed formulae with hydrocolloids such as starch or locust bean gum that develop a high viscosity in the stomach. Infants that are born premature, conventionally defined as low birth weight (LBW), require a special diet in order to survive and achieve the growth and development rates of normal infants. In hospitals, LBW infants may be fed formulae that contain greater amounts of protein, vitamins, minerals and calories than standard infant formulae to address the high nutrient needs and rapid growth of these infants. A 'post discharge formula' (PDF) is available for the LBW infant leaving the hospital environment. This is a nutrient-enriched formula generally intermediate in composition between preterm and term formulae (Lucas et al. 2001; Carver et al. 2001). Generally, the milk of a mother that has delivered a preterm infant is more nutrient dense than regular term milk. The milk may not however be able to support growth

Table 8.1 Typical composition (per 100 mL) of the main categories of infant formulae

Formula type	Energy, kcal	Protein, g	Fat, g	Carbohydrate, g
First Age (whey dominant)	66–68	1.3–1.7	3.4–3.9	7.0–7.6
First Age (casein dominant)	65–69	1.5–1.7	3.4–3.8	7.0–7.5
First Age (soy protein based)	65–68	1.8	3.6–3.7	6.7–6.9
'Follow on'	65–70	2.1–2.3	3.0–3.6	6.6–8.0
Low Birth Weight Growing up milk	80 75	1.9–2.4 2.5–3.5	4.2–4.4 3.0–3.5	7.9–8.6 8.5–10
Bovine Milk	66	3.2–3.8	3.7–4.4	4.8–4.9

The composition of bovine milk is given for reference

at the intrauterine rate and it may be beneficial to enrich the milk with a nutritional preparation known as 'human milk fortifier' (HMF). HMF formulae supplement human milk with protein, minerals, in particular calcium, and vitamins.

When the infant has been introduced to some solid foods, at ~4–6 months of age, a 'follow-on' formula may be provided as a complimentary food source. Follow-on formulae generally contain more protein, less fat and more carbohydrate that standard first age formula (Table 8.1). Follow-on formulae are designed to provide superior nutrition than bovine milk. The growing up or, toddler years, are critical periods of physical development and are characterized by variable dietary patterns. Specially formulated milks, commonly referred to as 'growing up milks' are available for young children in the age range 1–3 and 3–7 years. Some children aged 1 year and above are fussy or 'picky' eaters and may benefit from a specially designed milk that is supplemented with nutrients that fussy eaters typically lack. Additionally, nutritional preparations for pregnant and lactating women are available in different presentations such as multi-vitamin and mineral tablets or capsules, or beverages enriched in these nutrients.

National and international regulations and guidelines have been designed to ensure a safe and adequate nutritional intake for infants and children fed these nutritional products. Furthermore, limits have been set on the amount of processing aids, including emulsifiers that may be used during the manufacture of these products. The regulations and guidelines on permitted emulsifiers will be discussed below.

8.3 Emulsion Formation and Stabilization

Processes used to manufacture infant formulae are based on the concept that the products must be nutritionally adequate and microbiologically safe for infants to consume. Thus, steps that eliminate or restrict microbiological growth are central to production processes. The processing technology for each specific formula is proprietary to the manufacturer but, in general, it involves the preservation of an o/w emulsion by dehydration in the case of powder products or, sterilization in the case of ready-to-feed or concentrated liquid products.

Infant formula powder may be produced using three general types of processes. One process involves dry blending dehydrated ingredients to constitute a uniform formula. An alternative process involves hydrating and wet-mixing the ingredients and then drying the resultant mixture, usually by spray drying. In another process, which involves a combination of the two processes described above, a base powder is first produced by wet-mixing and spray drying the fat and protein ingredients and then dry blending the remaining ingredients (carbohydrate, minerals and vitamins) to create a final formula. The reader is referred to Pisecky (1997), Masters (2002) and Schuck (2002) for more details on the fundamentals and practice of spray drying.

Liquid formulae are available in a ready-to-feed format or as a concentrated liquid, which requires dilution, normally 1:1, with water. The manufacturing processes used for these products are similar to those used in the manufacture of recombined milk which has been reviewed extensively in the literature (Zadow 1982; Kieseker 1983; Sjollema 1987).

The formation of a stable o/w emulsion in which the fat or oil phase is uniformly distributed throughout the product is a common pre-requisite of both the powder and liquid production processes. In the case of dry blended formulae, the fat is already emulsified within a carrier system, usually one or more of the protein sources. In the case of liquid products and products prepared by the wet-mixing/spray drying system, a fluid fat blend is dispersed and emulsified in an aqueous system consisting of the proteins, carbohydrates and other minor ingredients such as minerals, vitamins and processing aids including emulsifiers. The mixture is then homogenised to form a uniform mixture with small fat droplets (typically <1 μm).

Homogenisation is normally achieved in dairy processes, including the production of infant formulae, by conventional valve homogenization in which fat globules are forced through a small orifice under high pressure. The combination of shear forces and impact forces significantly reduces the fat globule size. Microfluidization is an alternative homogenizing process, whereby the mixture enters an interaction chamber, which has fixed-geometry micro-channels that divides the product into tiny streams. The streams flow through the interaction chamber, accelerating to very high velocities. The streams collide with each other and under these conditions shear and impact forces create very small particles. A cooling coil may or may not be present after the interaction chamber. Homogenisation with a Microfluidizer® is usually performed at higher pressures than is used in conventional valve homogenisation (Olson et al. 2004) and has been successfully employed in processing of infant formulae (Pouliot et al. 1990).

After the formation of the o/w emulsion, it undergoes sterilization or dehydration to inactivate microorganisms. Thus, the emulsion formed must be capable of withstanding the associated pumping, shearing and thermal treatments. Depending on the process, the treatment may range from a typical high-temperature/short time heat treatment to pasteurize to a ultra-high-temperature (UHT) treatment (e.g. 135–150 °C for 3–5 s) to sterilize the mix. Generally, the emulsion is evaporated in a multi-effect continuous system at 40–70 °C to increase the solids content of the emulsion to ~50–58% (w/w) prior to spray drying. The final step involves the dehydration of the product until a low moisture content, typically <3%, is achieved. Spray drying is a common large scale drying system used to dry heat sensitive pow-

ders such as infant formula. The emulsion is atomized into minute droplets that fall through the drying chamber concurrently with hot air. Evaporation of water from the droplets takes place rapidly due to the large surface area. The resultant powder particles are conveyed and filled into containers such as cans or flexible pouches. Apart from the nutritional and microbial quality, the dehydrated emulsion must be easy to reconstitute in lukewarm water and when reconstituted must be free of lumps and other defects such as free fat, greasiness and white flecks that adhere to the sides of the feeding containers which are typically, clear plastic bottles. Sliwinski et al. (2003) studied the effects of spray drying on the properties of emulsions (20%, w/w, soybean oil; 2.4%, w/w, protein) prepared from SMP alone, WPI alone or SMP/WPI blends. Spray drying and reconstitution lead to a slight increase in the fat globule size of casein-dominant emulsions and a greater increase for the whey dominant emulsions.

In the production of liquid infant formulae, the o/w emulsion must be sterilized. This is achieved by thermal treatments such as UHT processing (e.g. 135–150 °C for 3–5 s) or in-container retort sterilization (e.g. 120 °C for 5–10 min) or a combination of these processes. Thus, the emulsion must be sufficiently heat-stable to withstand such severe thermal processes. The heat stability of the emulsions is closely related to the heat stability of the protein system and dependent on formulation variables such as the amount of protein and the sources used, fat content, pH and ionic strength (McSweeney et al. 2004; McCarthy et al. 2012).

If the emulsion is not sufficiently heat stable, fat globule aggregation occurs on heating as a result of interfacial protein-protein reactions to form clusters of fat globules. These fat globule aggregates, typically in the range 10–100 μm or larger, cream rapidly and thus, shelf life is reduced (McSweeney et al. 2004). The installation of an aseptic homogeniser after the UHT step is an effective way to disrupt these fat globule aggregates. In extreme cases, if the emulsion is not sufficiently heat stable to survive the thermal processing, the product coagulates upon sterilization.

8.4 Emulsifying Ingredients in Infant Formulae

The emulsifiers that are used in the production of infant formulae may be classified into two general categories; the proteins and the non-protein emulsifiers. The non-protein emulsifiers together with the hydrocolloids are usually classed in regulations as food additives.

8.4.1 Protein-Based Emulsifiers

A list of ingredients that are common protein sources in infant formulae is outlined in Table 8.2. Bovine milk proteins are widely used in the production of infant formulae.

Table 8.2 Protein ingredients commonly used in infant formulae

Name	Typical application
Skim milk powder	Infant formulae, follow-on formulae
Demineralised whey	Infant formulae
Whey protein concentrate	Infant formulae, follow-on formulae
α-Lactalbumin enriched/β-lactoglobulin reduced whey protein concentrate	Infant formulae Lactose free infant formulae
Milk protein isolate Soy protein isolate	Infant formulae for infants intolerant of dairy proteins
Partially and extensively hydrolysed proteins (whey protein, casein, soy)	Hypoallergenic infant formulae
Sodium-, Calcium Caseinates	Infant formulae, follow-on formulae

Adapted (i.e. whey protein dominant) first-age infant formulae are generally based on a combination of skim milk and whey protein. Demineralised whey, prepared by nanofiltration, electrodialysis or by ion exchange chromatography or some combination of these methods, or whey protein concentrates prepared by membrane separation techniques, are common whey protein sources. It has long been recognised that the levels of the individual whey proteins in human and bovine milk are quite different. Human milk contains higher levels of α-lactalbumin, lactoferrin and other minor whey proteins, such as secretory immunoglobulin A, than bovine milk. In addition, β-lactoglobulin the most abundant whey protein in bovine milk is absent from human milk. This has led to the development of protein fractions enriched in proteins abundant in human milk, such as α-lactalbumin, lactoferrin, osteopontin, and milk fat globule membrane proteins for use in infant formulae (Lein 2003; O'Callaghan and Wallingford 2002; Lönnerdal 2014).

Lactose-free formulations are based on milk protein- and whey protein concentrates or isolates from which the lactose has been removed by membrane filtration or enzymatic hydrolysis. Formulations devoid of dairy proteins and lactose are based on isolated soy protein. The protein source, soy protein isolate, typically contains 80–90% protein. The production of ingredients for formulae based on partially or extensively hydrolysed proteins generally involves enzymatic hydrolysis of proteins (casein, whey protein or soy protein) to peptides of low molecular weight followed by ultrafiltration to remove unhydrolysed protein and large polypeptides. Elemental formulae contain free amino acids and are devoid of protein or peptides. The non-protein emulsifiers are the main emulsifying agents in these products.

8.4.2 Non-protein Emulsifiers

The non-protein emulsifiers, or low molecular weight surfactants that are permitted in infant formulae, consumed in the EU are listed in Table 8.3. Scientific committees that advise on the types and levels of emulsifiers permitted in infant formulae work on the principle that it is prudent to keep the number of additives to the

Table 8.3 Emulsifiers permitted in EU infant formulae (as authorised by Regulation (EC) No. 1333/200815)

E. No.	Name	Maximum level (mg/L or mg/kg as appropriate)	Restrictions/exceptions
E322	Lecithins[a]	1000	
E471	Mono and diglycerides[a]	4000	
		5000	Infant formulae and follow-on formulae for special medical purposes
E472c	Citric acid esters of mono- and diglycerides of fatty acids[a]	7500	When sold as powder
		9000	When sold as liquid where the products contain partially hydrolysed proteins, peptides or amino acids
E473	Sucrose esters of fatty acids[a]	120	Only products containing hydrolysed proteins, peptides or amino acids
E1450	Starch sodium octenyl succinate	20	Infant formulae and follow-on formulae for special medical purposes

[a]If more than one of the substances E322, E471, E472c and E473 are added to a foodstuff, the maximum level established for that foodstuff for each of those is lowered with that relative part as is present of the other substance in that foodstuff

minimum necessary (Scientific Committee for Food, European Commission 1994). It is not surprising that the list of emulsifiers permitted is not extensive given that a considerable amount of safety data must be generated to prove safety of an emulsifier in infant formulae.

Upper limits for the food additives are established after considering factors such as acceptable daily intakes (ADIs) and technological requirements. In regular infant- and follow-on formula, the intact dairy or soy proteins are efficient emulsifiers and only limited levels of two emulsifiers (lecithin (E322) and mono-diglycerides (E472a)) are permitted (Table 8.3). However, in the case of products containing hydrolysed proteins, peptides or free amino acids, the use of non-protein emulsifiers is necessary to stabilize the emulsion. This is reflected in a more extensive list of permitted emulsifiers in these speciality infant formulae (Table 8.3). Thus, in addition to lecithin and mono-di-glycerides, citric acid esters of mono-di-glycerides of fatty acids also known as CITREM (E472c), sucrose esters of fatty acids (E473) and/or starch sodium octenyl succinate (E1450) may be used in certain types of formulae. Another emulsifier, not listed in Table 8.3 is 'mono- and di-acetylated tartaric acid esters of mono- and diglycerides' (E472e) (also known as DATEM), is approved for use in special infant formulae based on crystalline amino acids (FSANZ 2000; Canadian Food and Drugs Act 2003).

Lecithin is widely used as an emulsifier in the food industry. Vegetable-based lecithin is a by-product of vegetable oil processing. Soy lecithin, from soybean oil is the most widely used surfactant ingredient in the food industry (Stauffer 1999). It is a crude mixture of phospholipids, glycolipids, triglycerides, carbohydrates and traces

of sterols, free fatty acids and carotenoids. Crude mixtures from different geographical regions may be blended to give a consistent phospholipid composition and functionality. Lecithin may be modified enzymatically through hydrolysis or chemically by hydroxylation, aceylation or hydrogenation. The neutral lipids, mainly triglycerides are soluble in acetone and thus may be removed from the crude lecithin mixture to yield a product enriched in the polar lipids (phospholipids and glycolipids) by a process known as de-oiling. The production of lecithin fractions with a certain phospholipid profile is possible due to the differences in the solubility of the individual phospholipids in ethanol. Lecithin may also be isolated from egg usually by a combined extraction with ethanol and acetone (Bueschelberger 2004). The phospholipids in vegetable-based lecithin are primarily phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA) but only PC and PE predominate in egg lecithin. Egg lecithin is often used in the production of infant formula, as it is also a source of the long-chain polyunsaturated fatty acids arachidonic acid and docosahexaenoic acid. Natural lecithins have intermediate hydrophile-lipophile balance (HLB) values of ~8 (McClements 2005).

Mono-di-glycerides are produced by the interesterification of triglycerides with glycerol at high temperatures (200–250 °C) under alkaline catalysis. Commercial grade mono-di-glycerides are typically a mixture of 45–55% mono-di-glycerides, 38–35% diglycerides, 8–12% triglycerides and 1–7% free glycerol (Moonen and Bas 2004). Mono-di-glycerides are oil-soluble surfactants with relatively low HLB values (McClements 2005) and are widely used in the formulation of dairy emulsions (Dickinson 1997).

CITREM is formed by the esterification of citric acid and fatty acids with glycerol or by the reaction of a mixture of mono- and diglycerides with citric acid. This emulsifier is dispersible in hot water and soluble in edible oils. It is an ionic oil-in-water emulsifier (Gaupp and Adams 2004).

DATEM is formed by the esterification of mono- and di-acetyl tartaric acids and fatty acids with glycerol. The emulsifier is soluble in hot and cold water and partially soluble in warm oils. DATEM is an ionic o/w emulsifier and is more hydrophilic than its constituent mono- and di-glycerides (Gaupp and Adams 2004).

Sucrose esters of fatty acids are non-ionic compounds synthesised by the esterification of fatty acids or natural glycerides with sucrose. The emulsification properties are dependent on the type of fatty acid that is reacted with sucrose. Emulsifiers that span the hydrophilic-lipophilic (HLB) from 1 to 16 can be formed by reacting fatty acids in the C_8–C_{22} range with sucrose. Relatively hydrophilic emulsifiers (for use in w/o emulsions) can be produced by reacting short chain fatty acids with sucrose and relatively lipophilic emulsifiers (for use in o/w emulsions) can be produced by reacting long chain fatty acids, most commonly palmitic ($C_{16:0}$), oleic ($C_{18:0}$) or stearic ($C_{18:1}$), with sucrose (Nelen and Cooper 2004). These emulsifiers are tasteless, odourless and display a capacity to inhibit microbial growth (Fontecha and Swaisgood 1994).

Starch octenyl succinate anhydride (OSA Starch) is made by treating starch with the hydrophobic *n*-Octenyl succinic anhydride at pH 8–8.5. This starch derivative is anionic due to a carboxyl group and hydrophobic due to the C_8 unsaturated alkene chain. OSA starch is highly soluble in water, forming an opaque suspension (Viswanathan 1999). The synthesis, characterization and

structure of starches modified by octenyl succinic acid were reviewed by Sweedman et al. (2013).

8.5 Stabilizing Agents Used in Infant Nutritional Products

As with emulsifiers, hydrocolloids are regulated as food additives. The hydrocolloids that are permitted in infant formulae, for consumption within the EU are listed in Table 8.4. Further to this list, starch may be used as a source of carbohydrate and is permitted up to a maximum level of 0.2 g/L and 30% of the total carbohydrate in infant formula.

Table 8.4 Hydrocolloids permitted in EU infant formulae (as authorised by Regulation (EC) No. 1333/200815)

E. No.	Name	Maximum level (mg/L or mg/kg as appropriate)	Restrictions/exceptions
E412	Guar Gum[a]	1000 1000 10,000	Only where the liquid product contains partially hydrolysed proteins Follow on formula From birth onwards in products in liquid formulae containing hydrolysed proteins, peptides or amino acids
E440	Pectins	5000 10,000	In acidified follow-on formulae only From birth onwards in products used in cases of gastro-intestinal disorders
E407	Carrageenan[a]	300	Follow on formula
E410	Locust Bean Gum[a]	1000 10,000	Follow on formula From birth onwards in products for reduction of gastro-oesophageal reflux
E401	Sodium alginate	1000	From 4 months onwards in special food products with adapted composition, required for metabolic disorders and for general tube feeding
E405	Propane 1,2-diolaginate	200	From 12 months onwards in specialised diets intended for young children who have cow's milk intolerance or inborn errors of metabolism
E415	Xanthan gum	1200	From birth onwards for use in products based on amino acids or peptides for use with patients who have problems with impairment of the gastro-intestinal tract, protein malabsorption or inborn errors of metabolism
E466	Sodium carboxy methyl cellulose, cellulose gum	10,000	From birth onwards in products for the dietary management of metabolic disorders

[a]If more than one of the substances E407, E410 and E412 is added to a foodstuff, the maximum level established for that foodstuff for each of those substances is lowered with that relative part as is present of the other substances together in that foodstuff

Starches and gums may be chemically or enzymatically modified to insert a lipophilic group. For example, alginic acid may be esterified with propylene glycol to yield propylene glycol alginate (E405). Other regulatory agencies such as Codex Alimentarius permit the modified starches including distarch phosphate (E1412), acetylated distarch phosphate (E1414), phosphated distarch phosphate (E1413) and hydroxyl propyl starch (E1400) to first age infant formula.

8.6 Emulsifier Functionality in Infant Nutritional Products

8.6.1 Aspects of Stability

Infant formulae must meet stringent quality criteria concerning nutrient composition, microbiology, sensory (colour, mouthfeel, odour, taste) and appearance. Although emulsions are inherently unstable systems, nevertheless they can be manufactured to remain stable over the shelf-life of infant formula, generally 1–2 years for sterilized liquid emulsions and 18 months to 3 years for powders. At the end of the shelf life the product must remain acceptable to the consumer.

Ready-to-feed infant formulae are susceptible to the same instability problems as recombined milks products and beverage emulsions. Common defects include greasiness or ‘oiling off’, creaming, fat flecks, ringing, phase separation, fat creep and sedimentation. ‘Oiling off’ refers to the formation of an oil slick or beads on the surface of the product and is due to non-emulsified fat. Steps should be taken to minimise creaming because it influences many product features. Early into shelf-life, gentle shaking may restore a homogenous product, but later into shelf life, the cream layer may break up into small fat flecks that float on the surface. Alternatively, the cream layer may form a solid clump, which may prove difficult to disperse. A fat ring or fat collar may remain on the side of the container after shaking. Fat may also ‘creep-up’ along the neck of the container to generate an undesirable appearance; this fat may also prove difficult to re-disperse upon shaking. Creaming may result in the formation of distinct phases that appear different; one towards the top of the product that is enriched in fat and is generally milky white and lower phases that are depleted in fat and more translucent in appearance. If the product contains insoluble minerals, a layer of sediment may form on the base over time. In the case of powder products, the dehydrated emulsion does not undergo significant changes throughout the shelf life and its reconstituted appearance will reflect the quality of the emulsion that was originally dried. Generally, creaming is not an issue as the product is consumed soon after reconstitution but if the emulsion was of a poor quality before drying, undesirable features such as free fat, ‘oiling off’, greasiness and white flecks will reemerge upon reconstitution.

Therefore, an understanding of the factors that influence the stability of infant formulae is required in order to develop products that display an excellent appearance and a lengthy shelf life.

8.6.2 *Emulsifier Functionality*

The function of emulsifiers in infant formulae is to facilitate the formation of a stable emulsion and to improve its stability. This is achieved during homogenisation process when the emulsifiers (both protein and non-protein types) diffuse to and adsorb at the newly formed fat droplets to form an interfacial film or membrane. The stability of each oil droplet is dependent on the nature and extent of its interaction with neighbouring droplets in the continuous phase, which in turn is determined by the conformation, structure, electrical charge and the mechanical and rheological properties of the interfacial membrane (Das and Kinsella 1990). The properties of the interfacial membrane will depend on the proportions of each type of surface active component and their surface active properties; initially the most surface active component predominates at the interface and low molecular weight surfactants generally displace proteins over time (Euston 1997).

At fluid/fluid interfaces proteins lose their tertiary structure, unfold, and rearrange so that hydrophobic segments of the polypeptide chain orient towards the oil phase and hydrophilic segments orient towards the aqueous phase, and eventually form a cohesive film around the fat droplet. The interfacial properties of proteins, in general, are described in a comprehensive review by Das and Kinsella (1990). The milk proteins are excellent emulsifiers because they are amphipathic molecules containing polar and non-polar regions. For general reviews on the emulsifying properties of milk proteins, see Dickinson (2001, 2004) and Singh (2011).

Regular infant formulae can rely to a large extent upon the inherent emulsification properties of intact milk proteins to form stable emulsions. However, formulae that contain hydrolysed proteins, peptides or free amino acids, especially in a ready-to-feed format, require non-protein emulsifiers to create stable emulsions. These low molecular weight surfactants consist of a hydrophilic 'head' group and a lipophilic 'tail' group (Hasenhuettl 1997; Faergemand and Krog 2003; McClements 2005). The head group may be non-ionic (e.g. mono-di-glycerides, sucrose esters of fatty acids), anionic (e.g. CITREM, DATEM) or zwitterionic, containing both positive and negative charges on the same molecule (e.g. lecithin) (McClements 2005). The tail group usually consists of one or more hydrocarbon chains. The non-protein surfactants adsorb at the oil-water interface with the hydrophilic head oriented towards the water phase and the hydrophobic head oriented towards the lipid phase. During homogenisation, the presence of non-protein surfactants leads to a more rapid reduction in interfacial tension than with milk proteins alone, which facilitates the formation of smaller droplets, and thus, an emulsion with increased stability towards creaming (Dickinson et al. 1989b).

The composition, structure and rheology of the adsorbed layer that is formed by a mixture of proteins and non-protein surfactants is usually quite different from that formed by proteins alone. Consequently, the competitive adsorption of proteins and non-protein surfactants, the displacement of protein by non-protein surfactants and the interaction of non-protein surfactants with interfacial protein, are topics that have been extensively researched. A brief description of the underlying thermody-

namic reasons for the competitive adsorption behaviour of proteins and surfactants has been given previously (Joos and Serrien 1991). In most cases, the competitive adsorption of protein and non-protein surfactants reduces the protein surface coverage at the o/w interface (de Feijter et al. 1987; Courthaudon et al. 1991; Dickinson et al. 1993b; Euston et al. 1995). The mechanism of competitive protein displacement from globule interfaces has been studied (Mackie et al. 2000; Woodward et al. 2009). During the initial stage of protein displacement from the interface, emulsifiers access the globule interface through small gaps in the interfacial protein layer and can increase in concentration at the interface without necessarily displacing protein. A high concentration of adsorbed emulsifier can be present without inducing protein displacement (Wilde 2004) but eventually, the interfacial protein layer collapses as it can no longer withstand the surface pressure and the protein molecules move from the interface into the serum phase. The interfacial film may be rendered stronger or weaker than with proteins alone because of surfactant/protein competition. The amount of protein displaced depends on surfactant type and concentration, time, and environmental factors such as temperature. As a rule, non-ionic water-soluble surfactants (e.g. sucrose esters) displace more proteins from the interface than are non-ionic oil-soluble emulsifiers (e.g. mono-di-glycerides) (Oortwijn and Walstra 1979; Dickinson and Tanai 1992; Dickinson et al. 1993a, b, c; Dickinson 1995; Euston et al. 1995; Munk et al. 2014). Some non-protein surfactants interact and form complexes with proteins at the interface without necessarily displacing them (Doxastakis and Sherman 1984).

Non-protein surfactant emulsifiers can also interact with proteins adsorbed at the interface and non-adsorbed proteins in the aqueous phase. Dickinson (1993) described the binding of charged ionic surfactant molecules with protein as occurring in two separate phases. Initially, the polar region of the surfactant binds to specific charged sites on the protein surface, such as cationic regions owing to the presence of Lys, His or Arg residues and the non-polar section of the surfactant binds to hydrophobic regions on the protein surface. Then, the protein unfolds to expose its hydrophobic interior and hence further binding sites for the hydrophobic section of the surfactant. Non-ionic surfactants, on the other hand, exhibit non-specific hydrophobic interactions (Dickinson 1993). Several studies have demonstrated that surfactants interact with dairy proteins (Brown et al. 1982, 1983; Fontecha and Swaisgood 1994, 1995; Sarker et al. 1995; Antipova et al. 2001; Deep and Ahluwalia 2001; Istarova et al. 2005).

As well as determining the composition, structure, thickness, rheology and charge of the interfacial layer, the non-protein surfactants influence the properties of emulsions in other ways. Dickinson et al. (1989a) described some mechanisms that explain how non-protein surfactants influence the stability of dairy emulsions. Certain non-protein surfactants such as mono-di-glycerides affect fat crystallization and crystal structure in emulsion droplets (Euston 1997), which may destabilize the o/w emulsions (Boode and Walstra 1993). The non-protein surfactants influence the viscosity of the aqueous phase through the formation of self-bodying mesophase structures (Dickinson et al. 1989a). The nature of the interfacial membrane also influences the susceptibility of the emulsion to fat oxidation (Let et al. 2007; Horn et al. 2011).

The influence of surfactants on heat stability is of particular relevance to the manufacture of heat-sterilized recombined milk based beverages such as ready to feed infant formulae.

8.7 Functional Properties of Proteins as Emulsifiers

8.7.1 Emulsifying Properties of Non-hydrolysed Milk Protein Sources

The emulsifying characteristics of many of the individual caseins, in particular β-casein, have been studied in model emulsion systems (Dickinson et al. 1988, 1993a, b; Courthaudon et al. 1991; Leaver and Dalgleish 1992; Brooksbank et al. 1993; Dalgleish 1993; Atkinson et al. 1995; Leermakers et al. 1996). Similarly, the emulsifying characteristics of the individual whey proteins, including β-lg, α-lac and BSA have been studied (Dickinson and Matsumura 1991; Dickinson and Gelin 1992; Eaglesham et al. 1992, 1994; Dickinson and Iveson 1993; Dickinson et al. 1993a, b, c; Atkinson et al. 1995). Some important, emulsion-related, characteristics of the milk proteins are listed below:

- The individual caseins are relatively unstructured proteins with an amphipathic nature and thus, have high surface activities.
- The whey proteins are also amphipathic but in contrast to the caseins feature a globular structure and generally diffuse slower than the caseins to the o/w interface.
- Whey proteins form more viscous interfacial films than caseins (Boyd et al. 1973).
- Caseins preferentially adsorb at the o/w interface over whey proteins during homogenisation in emulsions prepared with skim milk (Oortwijn and Walstra 1979, 1982; Britten and Giroux 1991; Sharma and Dalgleish 1993; Sharma and Singh 1998; Brun and Dalgleish 1999; Dalgleish et al. 2002).

The proteins used in studies of simple model emulsions quite often consist of one protein type that is in the native form, whereas commercially available ingredients consist of many individual protein types that may be denatured during the isolation or manufacture of the ingredient or, denatured during the manufacture of the nutritional product. Therefore, some studies have focussed on complex food-type emulsions containing commercially available milk protein ingredients including those used in the production of infant formulae (Britten and Giroux 1991; Sharma and Singh 1998; Euston and Hirst 1999, 2000; Sourdet et al. 2002; McSweeney et al. 2004; Sarkar and Singh 2016).

Protein structure and flexibility are known to have an important influence on the emulsifying ability of milk protein ingredients. The caseins in micellar casein products such as skim milk powder (SMP) and milk protein concentrate (MPC) exist as colloidal particles; casein micelles, which are composed of individual submicelles linked together by calcium bridges. Non-micellar casein (as found in products such as sodium caseinate or total milk proteinate) and the globular whey proteins, as found in whey protein concentrates (WPC), may be considered as flexible proteins

that can readily unfold to form an interfacial film. Micellar casein behaves differently at interfaces to non-micellar casein and whey proteins. The calcium bridges restrict the extent to which casein micelles unfold at fluid/fluid interfaces and thus, the effective number of protein "particles" available for adsorption is lower for micellar casein than for non-micellar casein. Furthermore, there may also be a reduced tendency for micellar casein to adsorb at interfaces as the more hydrophobic groups are located at the core of the micelles, and the surface of the micelle is not very hydrophobic (Dalgleish 1996). Nevertheless, micellar casein can accumulate at the o/w interface by dissociating into submicelles (Courthaudon et al. 1999; Walstra et al. 1999). In general, a protein in the micellar or aggregated state forms an emulsion with a higher surface coverage, a higher surface viscosity and greater adsorbed layer dimensions than protein in the non-aggregated state such as non-micellar casein or globular whey proteins (Oortwijn and Walstra 1979). Mulvihill and Murphy (1991) found that micellar casein and calcium caseinate were not as surface active as sodium caseinate, but the micellar casein products formed more stable emulsions than sodium caseinate. Sharma and Singh (1998) found that emulsions (4%, w/w, fat), prepared using skim milk powder (SMP) had higher protein concentrations (~6 mg/m^2) at the interface than emulsions prepared using sodium caseinate or whey protein isolate (WPI) (~2 mg/m^2). The addition of WPI reduced the surface protein concentration in SMP-stabilized emulsions but had no effect on sodium caseinate stabilized emulsions. Euston and Hirst (1999, 2000) found that for a given protein concentration, non-aggregated caseinate and whey proteins facilitated the formation of o/w emulsions (20%, w/w, oil) with a finer range of droplet sizes than for aggregated caseins products such MPC and SMP. However, the emulsions made from MPC and SMP had a higher surface coverage and were less susceptible to creaming than emulsions made using caseinate. Caseinate-stabilized emulsions can exhibit depletion flocculation (Dickinson et al. 1997); at a certain concentration the non-adsorbed casein in the emulsions forms micelle-like aggregates which in turn causes depletion flocculation which accelerates creaming (Euston and Hirst 1999).

The extent of thermal processing during the manufacture of milk protein products can influence their emulsifying properties, particularly if the heating results in whey protein denaturation. Upon heating to >70–75 °C, whey proteins denature and the surface activity of the aggregates of denatured proteins is largely unknown and dependant on the process conditions used during manufacture such as temperatures, duration of heating, pH and ionic strength. Mellema and Isenbart (2004) studied the effect of heating milk proteins (WPC, SMP) on the rheological properties of o/w interfaces. It was found that preheating (85 °C for 20 min) a WPC solution (0.7%, w/w) resulted in denaturation and aggregation but the aggregates formed were surface active since denatured whey proteins are not stable in solution and tend to aggregate or adsorb. The interfacial properties of SMP were largely unaffected by preheating (45 or 85 °C for 20 min) or by the type of powder used (low, medium or high heat SMP).

Infant formulae that have an increased ratio of W:C compared to bovine milk, are formulated by combining whey protein sources with casein sources in the appropriate ratios. The emulsifying properties of whey protein and casein blends have been stud-

ied (Britten and Giroux 1991; Sourdet et al. 2002). Britten and Giroux (1991) found that as the whey protein:casein (W:C) ratio in emulsions (30%, w/w soya oil; 1%, w/w, protein) increased, the surface protein concentration decreased. The protein sources used were sodium caseinate alone, WPI alone or sodium caseinate/WPI blends. Emulsions containing casein alone were the most susceptible to creaming and coalescence. The extent of emulsion destabilization decreased when the protein solutions were heated (80 °C × 30 min) before emulsion formation. Sourdet et al. (2002) reported that emulsions (9%, w/w, palm kernel oil), prepared using WPI as the sole protein source had a lower protein surface coverage than similar emulsions prepared using a SMP/WPI blend (60:40 W:C ratio) or SMP alone. Furthermore, emulsions containing WPI alone had aggregates of fat globules, whereas WPI/SMP-containing or, SMP-containing emulsions had fat globules with a narrow, mono-modal particle size distribution. In the study by Sliwinski et al. (2003), it was found that the amount of adsorbed protein present in emulsions (20%, w/w, soybean oil; 2.4% protein) prepared from SMP alone, WPI alone or SMP/WPI blends was not affected by spray drying and subsequent reconstitution. However, characterisation of the interfacial proteins showed that while the composition of the adsorbed layer of casein-dominant emulsions was largely unaffected by spray drying and reconstitution, emulsions containing between 50–90% whey protein had increased levels of whey protein at the interface after spray drying and reconstitution, even though the amount of adsorbed protein did not change, i.e. casein was displaced by whey protein. The authors postulated that non-adsorbed caseins could prevent the adsorbed caseins from being displaced by aggregating whey proteins in the casein-dominant emulsions.

Recently, novel milk protein fractions, such as α-lactalbumin (α-lac) and lactoferrin enriched whey protein fractions, have been developed especially for use in infant nutritional products. These fractionated ingredients may have different emulsifying proteins to unfractionated whey protein. It has been demonstrated that β-lg is more surface active than α-lac (Yamauchi et al. 1980; Srinivasan et al. 1996; Sharma and Singh 1998). Lam and Nickerson (2015) studied the effect of pH and temperature pre-treatments on the structure, surface characteristics and emulsifying properties of α-lac and found that changes to protein conformation using various pH and temperature pre-treatments influenced the proteins adsorption properties at the oil–water interface. Horn et al. (2013) studied lipid oxidation in 10% fish oil-in-water emulsions prepared with whey protein isolates with varying ratios of α-lac and β-lg. The results showed that, at pH 7 the emulsifier with the highest concentration of β-lg protected more effectively against oxidation during emulsion production, whereas the emulsions with the highest concentration of α-lac were most stable to oxidation during storage. These differences were attributed to differences in the pressure and adsorption induced unfolding of the individual protein components. Lactoferrin, is another protein, enriched in human milk. At neutral pH, it has a net positive charge (Shimazaki et al. 1993) a unique property that may be exploited in stabilizing o/w emulsions (Ye et al. 2012; Schmelz et al. 2011; Ye and Singh 2007). McCarthy et al. (2014) reported that emulsions containing β-casein at the interface were destabilized when calcium chloride was added due to calcium bridging of negatively charged phosphate groups. However, emulsions containing lactoferrin were stable against calcium induced aggregation as these oil droplets were positively charged.

8.7.2 *Emulsifying Properties of Hydrolysed Milk Protein Sources*

The emulsifying properties of hydrolysed proteins are related to the degree of hydrolysis (DH), the molecular weight distribution (MWD) and the amphiphilicity of the peptides formed (Rahali et al. 2000; Van der Ven et al. 2001; Euston et al. 2001b). The literature is somewhat ambiguous about the emulsion-forming ability of hydrolysates of casein and whey protein and the stability of resultant emulsions. Some studies have reported that the emulsion forming ability of low DH hydrolysates of casein (Haque and Mozaffar 1992; Chobert et al. 1988a, b) or whey protein (Haque and Mozaffar 1992; Vojdani and Whitaker 1994) is improved compared to the intact proteins that the hydrolysates were derived from but other studies have reported that the emulsion forming ability is reduced after hydrolysis of casein (Chobert et al. 1988a; Slattery and Fitzgerald 1998; Euston et al. 2001b). In general, intact milk proteins form more stable emulsions than their hydrolysates do (Haque and Mozaffar 1992; Agboola and Dalgleish 1996). Euston et al. (2001b) showed that emulsifying properties of hydrolysates of whey protein concentrate (WPC) were dependant on the degree of hydrolysis. Whey protein hydrolysates (WPH) with low DH values (4–10%) displayed poorer emulsifying ability than intact WPC. Hydrolysates with intermediate DH values (10–27%) showed improved emulsifying ability but hydrolysates with high DH values (27–35%) displayed poor emulsifying ability and emulsion stability. In a comparison of casein and whey protein hydrolysates prepared using commercially available enzymes, van der Ven et al. (2001) found that whey protein hydrolysates formed emulsions with bimodal droplet size distributions, indicating poor emulsion-forming ability while some casein hydrolysates demonstrated similar emulsion-forming ability to that of intact casein. The emulsion stability was related to the apparent molecular weight distribution of hydrolysates; emulsions formed using hydrolysates with a relatively high amount of peptides >2 kDa were more stable than emulsions formed using hydrolysates which contained smaller peptides. Lajoie et al. (2001) evaluated the role of cationic and anionic peptidic fractions, isolated from an ultrafiltered whey protein tryptic hydrolysate mixture by anion- or cation-exchange chromatography, as potential replacers of carrageenan in a model infant formula. The addition of the cationic peptidic fractions reduced emulsion stability compared to the control with carrageenan, whereas the creaming rate was reduced when the anionic peptidic fractions were used in the formulation. The properties of formula emulsions (4%, v/w, sunflower oil) prepared from whey protein isolate (WPI) or WPH at 3.7% and 4.9% (w/w) were investigated by Tirok et al. (2001). WPH-containing emulsions had a significantly higher mean droplet size were more susceptible to coalescence and creaming than WPI-containing emulsions. However, WPH-based emulsions could be stabilized against creaming and coalescence, when a low level of protein was used in combination with hydrolysed lecithin and glucose syrup.

8.7.3 *Emulsifying Properties of Soy Protein Sources*

Non-dairy infant formulae normally use soy protein isolate (SPI) as the protein source. The soybean proteins have traditionally been classified according to ultracentrifugal analysis into 2S, 7S, 11S and 15S fractions; the 7S (β-conglycinin) and 11S (glycinin) fractions are the predominant proteins (Aoki et al. 1980). The soy proteins are also amphipathic proteins containing both hydrophobic and hydrophilic amino acids and hence can act as emulsifiers. Mitidieri and Wagner (2002) and Palazolo et al. (2003) found that oil-in-water emulsions, stabilized using native SPI (at concentrations in the range 1–10 mg/mL) were very stable against coalescence but emulsions prepared with denatured SPI were unstable. These results were linked to nature of the interfacial protein layer formed; due to the compact globular structure and low surface hydrophobicity of the native SPI, a monolayer protein film formed around the oil droplets that sustained emulsion stability. The denatured SPI, on the other hand, formed a weak multilayer film that was susceptible to stress.

8.8 Functional Properties of Non-protein Emulsifiers

8.8.1 *Lecithin*

As lecithin has intermediate solubility characteristics and HLB number (~8), it is not particularly suitable for stabilizing either o/w or w/o emulsions when used in isolation (McClements 2005) but it may be effective when used in combination with other surfactants, such as proteins in the case of infant formulae.

The main surface-active components of lecithin, the phopholipids (PC, PE, PI and PA) each consists of a hydrophilic, or polar, head group and a hydrophobic tail group (the fatty acid chains). Thus, at o/w interfaces, polar head groups orientate towards the water phase and fatty acid chains orientate towards the lipid phase. As lecithin contains mostly unsaturated fatty acids, it is oil-soluble at ambient temperatures unlike the other widely used emulsifier in infant nutritional products, the mono-diglycerides, which must be melted at ~70 °C to solubilize in oil.

In the manufacture of infant nutritional products, lecithin is added primarily to improve emulsion stability. During emulsion formation and subsequent processing and storage, phospholipids influence emulsion properties through a combination of several factors including electrostatic and van der Waals forces, protein displacement and the formation of protein/phospholipid complexes. The net effect is a reduction in the interfacial tension (Yamamoto and Araki 1997) and oil droplet size (Dickinson and Iveson 1993; Sunder et al. 2001) and consequently, increased emulsion stability.

The inclusion of charged phospholipids at the o/w interfaces influences the electrostatic repulsion between oil droplets (Arts et al. 1994; Van Niewenhuyzen and Szuhaj 1998; Rydhag and Wilton 1981). The emulsion stabilizing effect of zwitter-

ionic phospholipids (PC, PE) is related to the formation of a lamellar liquid crystalline phase around the oil droplets, which causes a local viscosity increase, and the van der Waals attraction force between pairs of droplets is largely reduced (Friberg and Solans 1986).

The displacement of proteins by lecithin is complex due to variability in the head group and fatty acid chain types of the constituent phospholipids, the formation of a range of liquid crystalline phases in water and phospholipid/protein interactions (Dickinson 1997). In general, phospholipids are not very effective at completely displacing milk proteins from the o/w interface (Dickinson and Iveson 1993; Fang and Dalgleish (1996a, b). For example, Courthaudon et al. 1991 found that the addition of lecithin at high emulsifier: protein molar ratios (M_R) (>16) only lead to the partial displacement of protein from the interface of an o/w emulsion (0.4%, w/w, β-casein; 20%, w/w, oil). The competitive adsorption at the interface between proteins and lecithin is further complicated by the interaction of lecithin with adsorbed proteins and non-adsorbed proteins in the aqueous phase (Fang and Dalgleish 1993). Several studies have demonstrated an interaction of certain phospholipids with milk proteins in general (Korver and Meder 1974) or with specific proteins such as β-lg (Brown et al. 1983; Kristensen et al. 1997; Sarker et al. 1995). The combination of interfacial protein displacement (Courthaudon et al. 1991; Dickinson et al. 1993a) and the formation of protein/phospholipid complexes (Kristensen et al. 1997; Lefévre and Subirade 2001; Istarova et al. 2005) is significant in the production of thermally treated milk based products as an improvement in heat stability usually results. Thus, another reason for using lecithin in ready-to-feed infant formulae is to increase heat stability. Several studies have demonstrated that lecithin improves the heat stability of milk (Hardy et al. 1985; Singh et al. 1992; McCrae and Muir 1992), whey protein stabilized emulsions (Jimenez-Flores et al. 2005) and other dairy based products such as an artificial coffee creamer (Van der Meeren et al. 2005). Euston et al. (2001a) noted that at the initial stages of heating an o/w emulsion (1%, w/w, whey protein; 20%, w/w, soya oil) at 100 °C, low concentrations (<0.2%, w/w) of PC accelerated the rate of heat-induced aggregation of droplets, but as heating continued beyond 60 s, PC reduced the rate of aggregation. Emulsions containing 0.5% or 1% (w/w) PC proved resistant to heat-induced fat globule aggregation. In the same study, when glycerol monostearate (GMS) was included in the emulsion at 1% (w/w) the rate of heat-induced aggregation of fat globules was accelerated compared to the control with no emulsifier. A study by McSweeney et al. (2008) demonstrated that lecithin improved the heat stability of a model ready to feed infant formula.

Lecithin does not appear to be a particularly good emulsifier in emulsions containing hydrolysed proteins. A study by Tirok et al. (2001) may explain why this is the case. In the study, it was noted that emulsions (4%, w/w, sunflower oil) containing whey protein hydrolysate (3.7% or, 4.9%, w/w) and de-oiled soybean lecithin (0.48% or, 0.70%, w/w) rapidly destabilized. The results indicated that there was a preferential adsorption of lecithin over peptides and this may have resulted in a reduction in electrostatic and steric repulsion, thus, promoting coalescence. Normally, when a high concentration of non-protein emulsifier is used, multilayers of a lamellar liquid

crystalline phase increase stability (Dickinson 2001). However, the authors postulated that the presence of WPH peptides at the interface may have interfered with the formation of such an organized structure at the interface. A study by Drapala et al. (2015) on whey protein hydrolysate based oil-in-water (O/W) emulsions containing lecithin (0–5%, w/w, oil) found that lecithin decreased the interfacial tension between oil and aqueous phases of model emulsions and allowed formation of smaller oil droplets on homogenisation. However, on storage, low-intermediate levels (1–3%) of lecithin caused coalescence and a shift to a bimodal fat globule size distribution.

Lecithin may also be added to infant formula to improve powder wettability. Infant formula powder must reconstitute fully and quickly, however in common with other types of spray dried, fat-filled milk powder, it often has a high surface fat coverage which hinders wetting upon reconstitution. The reconstitution time depends on how quickly the powder particles wet, sink below the surface of the water, disperse and solubilize (Forny et al. 2011) and wetting is often the rate limiting step (Schubert 1993). The wettability of milk powders may be enhanced by coating powder particles with lecithin. To achieve this, lecithin may be added to the spray-drying feed line or during the agglomeration process (Millqvist-Fureby and Smith 2007; Hammes et al. 2015) with the aim of coating the surface of the powder milk particles with a surface active agent and thus, increasing their affinity for water. Several studies have shown that the addition of lecithin improves powder wettability (Lallbeeharry et al. 2014; Tian et al. 2014; Hammes et al. 2015).

Infant formula is typically supplemented with poly-unsaturated fatty acids and is thus, susceptible to lipid oxidation. The oxidative stability of oil-in-water (O/W) emulsions is highly dependent on the interfacial layer (Berton-Carabin et al. 2014). Berton et al. (2011) found that the protein-stabilized interfaces are less efficient at protecting emulsified lipids against oxidation than surfactant-stabilized interfaces. Studies have shown that lecithin can improve the oxidative stability of oils and oil-in-water emulsions (Nasner 1985; Hamilton et al. 1998; Judde et al. 2003; Horn et al. 2011; García-Moreno et al. 2014). Various antioxidative mechanisms have been proposed for phospholipids, including metal-chelating properties, the formation of an interface thus limiting contact between the lipids and atmospheric oxygen and synergistic effects with anti-oxidants (Haahr and Jacobsen 2008). A study by Zou and Akoh (2013) demonstrated that lecithin improved the oxidative stability of a model infant formula.

8.8.2 Mono-Di-Glycerides

Mono-di-glycerides are non-ionic oil-soluble surfactants and are the most widely used emulsifiers in the food industry (Zielinski 1997). As they are predominately hydrophobic and dissolve preferentially in oil, they are typically used to stabilise w/o emulsions. In the case of infant nutritional products, mono-di-glycerides are not particularly useful when used alone, but when used in combination with other surfactants, such as proteins and/or lecithin, mono-di-glycerides act to further reduce

the interfacial tension. This facilitates the formation of small oil droplets during homogenisation. Dickinson and Tanai (1992) have shown that the emulsion droplet size is reduced when mixtures of proteins and GMS are used as the emulsifiers. The formation of small oil droplets (<1 μm) is important to maintain the shelf-life stability of ready-to-feed or concentrated liquid infant formulae.

The disruption of adsorbed milk proteins by mono-di-glycerides has important implications for the processing and shelf life stability of emulsions. Mono-di-glycerides are known to partially displace milk proteins from o/w interfaces (Barfod et al. 1991; Krog and Larsson 1992; Gelin et al. 1994; Pelan et al. 1997; Davies et al. 2000, 2001; Munk et al. 2014).

- GMS displaced a significant proportion of adsorbed milk protein in a cream liqueur emulsion system (Dickinson et al. 1989b).
- Britten and Giroux (1991) found that the inclusion of commercial grade mono-di-glycerides in emulsions (30%, w/w, soya oil; 1%, w/w, protein) prepared from WPI alone, sodium caseinate alone or, blends of WPI and sodium caseinate with various W:C ratios, reduced the surface protein load.
- Davies et al. (2001) reported that at concentrations of 2 g/100 g in the oil phase, saturated mono-di-glycerides (glycerol monopalmitate (GMP) or GMS) displaced more protein from a sodium caseinate stabilised o/w emulsion than the unsaturated glycerol monoolein (GMO). This effect may be explained by the differences in the properties of adsorbed layers; the fatty acid chains of the saturated mono-di-glycerides may be able to align in more closely packed layers at the interface compared to the fatty acid chains of unsaturated mono-di-glycerides.

Following the displacement of proteins by low-molecular weight surfactants, the mechanical strength of the interface and the orthokinetic stability of protein-stabilised emulsions is reduced (Euston 1997). Mono-di-glycerides are very effective at displacing proteins from the interface, particularly at temperatures below ~15 °C. Upon cooling, mono-di-glycerides promote fat crystallization; emulsions with added mono-di-glycerides have higher solid fat content compared to emulsions with no added mono-di-glycerides (Davies et al. 2001; Miura et al. 2002). Saturated mono-di-glycerides (GMS, GMP) have a greater ability to initiate fat crystallization than unsaturated mono-di-glycerides such as GMO (Davies et al. 2001). The presence of fat crystals further promotes the destabilization of emulsions under shear; fat crystals protruding from the emulsion droplet may pierce the thin interfacial film thus promoting coalescence of neighbouring droplets. Mono-di-glycerides may promote both protein displacement and fat crystallization during the storage of infant formulae at low storage temperatures prior to the final thermal processing or dehydration step. The net effect may be to reduce the stability of the emulsion to shearing and turbulent forces. Protein displacement by mono-di-glycerides may also influence the thermal stability of emulsions. As mentioned above, Euston et al. (2001a) found that GMS promote the heat-induced aggregation of a whey protein stabilized emulsion. Similarly, McSweeney et al. (2008) showed that monoglycerides reduced the heat stability of a model infant formula emulsion; they postulated that the displacement of interfacial protein and as a consequence, reduced steric and

electrostatic repulsive forces in addition to conformational changes in the adsorbed protein layer may explain the negative impact of monoglycerides on heat stability.

8.8.3 Organic Esters of Mono-Di-Glycerides (CITREM and DATEM)

CITREM (E472c) and DATEM (E472d) are used in the production of infant formulae based on hydrolysed proteins, peptides or amino acids (Table 8.3). Generally, the degree of protein hydrolysis in these products is such that the emulsion must be stabilised entirely by non-protein emulsifiers. CITREM and DATEM are particularly suitable for use in o/w emulsions as they have high HLB values. Thus, at the interface, the fatty acid group orientates into the oil phase while the negatively charged organic acid groups extends into the aqueous phase stabilizing the emulsion through electrostatic repulsion. The electrostatic repulsion prevents coalescence and a reasonably long shelf life can be achieved.

CITREM was found to be a particularly good emulsifier in a model infant formula emulsion containing hydrolysed whey proteins (McSweeney 2007). An emulsion containing WPH alone had large fat globules, a high creaming rate and was susceptible to coalescence upon thermal processing. The impact of various emulsifiers, CITREM, lecithin, mono-di-glycerides and DATEM and their interactions was studied. CITREM was the only effective low molecular weight emulsifier to reduce fat globule size and to generate emulsions with low creaming rates and resistance towards coalescence and thermal processing. The inclusion of the other low molecular weight emulsifiers, lecithin, mono-di-glycerides and/or DATEM, along with CITREM did not result in any real benefits in terms of emulsion stability. In fact, mono-di-glycerides promoted coalescence in the model infant formula emulsions upon storage. The susceptibility to coalescence was explained in terms of displacement of WPH peptides and/or interaction between the low molecular weight emulsifiers and adsorbed and non-adsorbed WPH peptides with subsequent loss of steric stabilization.

8.8.4 Sucrose Esters of Fatty Acids

Sucrose esters of fatty acids (E473) may be used in the production of infant formulae based on hydrolysed proteins, peptides or amino acids (Table 8.3). At the interface, the fatty acid group(s) orientates into the oil phase while the sucrose groups extend into the aqueous phase. This group of emulsifiers is not widely used in the production of infant formulae and in general, there is a lack of information on the literature related to the use of these emulsifiers in fluid o/w emulsions.

8.8.5 Starch Octenyl Succinate Anhydride

When the starch octenyl succinate anhydride (OSA starch) macromolecule adsorbs at the o/w interface it stabilizes droplets against coalescence by steric hindrance and charge repulsion. Unlike proteins, the functionality of OSA starches are largely independent of the pH and ionic strength of the emulsion (Tesch et al. 2002). This is because the steric stabilization is the dominant force in OSA starches (Taherian et al. 2006), In a study, Tesch et al. (2002) demonstrated that OSA starches could replace whey proteins as emulsifiers in o/w emulsions and that unlike whey proteins, OSA starch stabilized emulsions were not susceptible to aggregation near the iso-electric point of the protein. Mahmoud (1987) reported that OSA starch was very effective in stabilizing a hypoallergenic formula based on extensively hydrolysed proteins. When comparing oil-in-water emulsions (5% rice bran oil, 10 mM citrate buffer) stabilized using whey protein isolate (WPI, 0.02–10 wt%) or an OSA starch (0.2–5 wt%), Charoen et al. (2011) found that the emulsions formed with the modified starch were more stable to external conditions (pH, salt and temperature) than emulsions made using WPI. Although a permitted ingredient in certain circumstances, OSA starch (E1450) is not a widely used ingredient in the production of infant formulae.

8.9 Function of Stabilizers

Traditionally, hydrocolloids such as gums and starches have been regarded as thickeners. Their stabilizing effect on emulsions derives from an increase in the viscosity of the aqueous phase. The kinetic motion of the droplets is reduced, resulting in a lower rate of flocculation and coalescence. As they are not true emulsifiers, they are not considered in this review.

8.10 Summary

Infant formulae are o/w emulsions that must maintain excellent stability throughout a long shelf life. These products are available in a ready-to-feed liquid format, as a concentrated liquid that requires dilution or, as a dehydrated powder that must be reconstituted prior to use. Regular infant formulae based on intact proteins may be stabilized by the proteins alone. Lecithin and mono-di-glycerides are non-protein emulsifiers that may be used to enhance the stability of these products, particularly, ready-to-feed or concentrated liquid products. Lecithin may also be added to improve the wettability of spray dried powders upon reconstitution. The use of emulsifiers to reduce the susceptibility of emulsions to lipid oxidation is another consideration. In addition to lecithin and mono-di-glycerides, other emulsifiers

(CITREM, DATEM, OSA starch and sucrose esters of fatty acids) and stabilizers are permitted for use in infant formulae that are based on hydrolysed proteins, peptides or amino acids. Apart from the emulsifiers used, the emulsion quality of infant formulae is influenced by other compositional variables; protein-stabilized emulsions are especially sensitive to pH and ionic strength effects (McClements 2004). Therefore, infant formulae are formulated not only to generate a target composition (label claim) but also to have pH values and ionic strengths that coincide with optimum emulsion stability (McSweeney et al. 2004). This is achieved by selecting appropriate sources and combinations of proteins and mineral salts. The stability of the emulsion formed is dependent on the conditions during the homogenization step (method, temperatures, pressure, number of passes) and unit operations that thermally process the emulsion such as the terminal sterilization step (McSweeney et al. 2004) or that dehydrate the emulsion (Sliwinski et al. 2003). Finally, emulsion quality is also influenced by environmental stress during transport and storage, such as temperature and mechanical agitation.

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Chapter 9
Current Emulsifier Trends in Dressings and Sauces

Bruce Campbell

9.1 Introduction

As a category, the technologies and principles used to create, characterize and produce dressings and sauces have been comprehensively reviewed (Ford et al. 1997; Sikora et al. 2008). Four major trends in the dressing and sauces category were identified (Ford et al. 1997) that have continued to the present. First is the continued interest in low-fat and fat-free dressing systems; second, an increasing breadth of new flavor options; third, the ongoing need to control production costs; and fourth, an intensified consumer interest in healthy food options with attention to nutrition and bio-active ingredients. More recently, a fifth trend of healthy perception has emerged with a crescendo of focus on cleaner ingredient lines demanding the removal of various additives such as artificial colors, flavors, preservatives, reducing sodium as well as the inclusion of ingredients supporting a "better for you" choice in the market place. This chapter addresses the impact of these trends on the selection of emulsifiers used to produce these products that have >5% oil, thus products such as vinaigrettes, soy sauce, spaghetti sauce, mustard, ketchup and barbecue sauces will not be discussed.

9.2 Dressing Stability

As oil-in-water emulsions, dressing manufacturing requires emulsifiers and shear processing to emulsify the oil phase into small oil droplets. The early emulsifier of choice was eggs and specifically egg yolks. Through the turn of the twentieth century, most dressings and sauces were produced for immediate consumption at home

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or restaurants where long shelf lives (>1–3 days) were not expected or needed. As a result, most dressings were produced in smaller batches (<10 lb) using raw materials with readily available processing/mixing equipment while taking into account local taste preferences. Under these circumstances dressing textures and flavors could vary greatly among different producers such as small food service companies and restaurants for any given dressing type. As technologies developed for extending product shelf-life in terms of physical stability and food safety, their incorporation enabled larger scale production and wider geographic product distribution. Variation in texture, mouthfeel, color/opacity, shelf-life stability and sensitivity to distribution channel conditions inherent with the ever increasing batch sizes and production locations led to stricter controls on ingredient quality and dressing manufacturing protocols.

As the dressings market matured through the 1980s, the dressing businesses increasingly depended on technological advances to maintain profitability and market share. These business pressures demanded reductions in ingredient/manufacturing costs without compromising product quality. Controlling ingredient costs became an irresistible opportunity to pursue through the reduction of oil, egg yolk solids and the replacement or lowering the levels of expensive gums. Furthermore, production cost reductions led to efforts to increase dressing production rates while consolidating slower and less efficient production lines. However, faster production rates subject dressing systems to more intense shear regimes originating from more intense mixing, faster flow rates, and shorter mixing times. More intense processing conditions tend to put both product stability and texture acceptability at risk.

Dressing manufacturers traditionally dealt with stability through a combination of ingredient selection, proportions, ingredient addition order as well as adjusting processing conditions to correct product specific stability issues. After standardizing their manufacturing conditions, there is usually little incentive for producers to develop in-depth technical understanding of the factors contributing to emulsion stability or instability. As long as dressing manufacturers have access to their trusted ingredient supply chains, any subsequent small formulation and processing changes rely on experienced operators to devise the appropriate “fix” to maintain product stability.

Even though reducing ingredient costs through reformulation grew profit margins, maintaining product quality attributes became challenging. Furthermore, additional formulation constraints were evolving from several health-wellness challenges concerning the dressing and sauces category beginning around the mid 1980s. The consumer desire for “better for you” and “good for you” options became significant drivers for formulation change. Most notably the excessive consumption of fat, saturated fat, trans-fat, cholesterol, sodium, led to a myriad of lower fat, no-fat, omega-3 enriched oils, reduced sodium, cholesterol-free dressing products. By 2010, the trend towards promoting fresher and more natural ingredient lines without artificial flavors, colors, and preservatives emerged and rapidly gained momentum through social media. Several dressing manufacturers reconsidered their ingredient options and gradually replaced some of their ingredients with more authentic/natural ingredients having consumer friendly names, organic sourcing, and limiting or

abolishing GMO (genetically modified organism) ingredients. Meeting these consumer needs through reformulation should invigorate the dressings and sauces category, however, these reformulation efforts may bring challenges for meeting product organoleptic and stability criteria. Regardless how close reformulated products are to parity to their respective standard formulations, market success still hinges on product stability.

9.2.1 Dressing Stabilization Mechanisms and Evaluation

Emulsion stability is essential for dressing performance followed by the impact, in no particular order, dressing mouthfeel, texture, flavor release and appearance. Emulsion stability depends on the robustness of the interfacial molecular structures and their interactions within an oil droplet interfacial layer as well as between the interfaces of neighboring oil droplets. Furthermore, emulsion oil droplet size and size distributions affect the rate and mechanism of emulsion destabilization as described in more detail by Tadros (2013). For a given emulsifier, emulsions with larger droplets coalesce with less force than smaller droplets. Also, interfacial aging and any accompanying structural changes will additionally affect the robustness of the oil-water interface. Therefore, the contributors to emulsion stability are both chemical and geometrical and the combination of interfacial chemical structures and droplet geometries of the interacting oil droplets ultimately define an emulsion's stability behavior under given storage conditions.

The primary emulsion destabilization events affecting dressings and sauces are oil droplet creaming rates and ultimately coalescence. Oil droplet creaming rates generally follow Stokes behavior shown in the following equation and presented by McClements (2015) in more detail.

$$V = \frac{d^2 g\left(\rho_p - \rho_s\right)}{18\eta}$$

This equation shows the droplet creaming rate (V) in a gravitational field (g) is inversely proportional to the continuous phase viscosity (η) while directly proportional to the density difference between oil and aqueous phase ($\rho_p - \rho_s$) and the square of the oil droplet diameter (d). Furthermore flocs of small droplets will tend to behave as a single fat droplet having the larger floc diameters. Over the course of creaming, oil droplets become concentrated near the top surface to form numerous inter-droplet contacts which increases the probability of oil droplet coalescence and the eventual formation of a free oil layer.

The Stokes equation shows the creaming rate is most affected by droplet diameter and thus reducing fat droplet diameter is the most effective way to reduce an emulsion's creaming rate. However, decreasing fat droplet size should be controlled to minimize any unintended or undesirable effects on an emulsion's appearance,

mouthfeel and flavor release profile. The Stokes equation shows creaming rates can also be diminished with increasing viscosity. An appropriate selection of hydrocolloid viscosifiers and dissolved solids can boost viscosity to further reduce the creaming rate.

The Stokes equation assumes the emulsion oil droplets behave independently because it does not account for droplet interactions during creaming. Emulsion creaming rates can be further reduced through increasing oil phase volumes which increases the number of oil droplets per unit volume and thus inter-droplet collisions for a given particle size distribution.

Decreasing the density difference between the oil and aqueous phases to zero should effectively stop oil droplet creaming regardless of droplet size and continuous phase viscosity. However, this is currently an impractical option due to the few available formulation options such as brominated triglycerides to increase oil density along with its low consumer appeal as well as lowering the level of dissolved solids to decrease the aqueous phase density which severely impact taste and possibly raises microbial risk.

The most practical approach to managing emulsion creaming rates is to both increase the continuous phase viscosity with an appropriate decrease in fat droplet size while maintaining the essential product features within acceptable limits of consumer preference. Instruments such as the LumiSizer® (LUM GmbH) and TurbiScan® (Formulaction SA) provide quantitative tools to evaluate prototype and product emulsion stability properties as impacted by various formulation and processing changes. The Turbiscan® directly measures emulsion separation rates without acceleration while the LumiSizer® measures these separation rates under accelerating conditions through centrifugation. Separation rate information can be used to estimate shelf life prior to measureable separation. Alternatively, product separation behavior can be evaluated with any preparative centrifuge. However, this approach only provides the extent of separation after a given amount of time spinning at a set spinning rate and thus shows which products separate to a greater or lesser extent under the specified conditions (rcf and time).

9.3 Dressing Emulsifiers

Emulsifiers in dressings are essential for producing the desired emulsion quality in terms of key dressing attributes such as appearance, flavor, texture and stability. Furthermore, emulsification parameters such as the oil addition rate, emulsifier continuous phase solubility as well as shear processing conditions will further impact oil droplet size distribution and subsequent product attributes including stability. Historically, food systems such as the dressing oil-in-water emulsion types have had limited food approved synthetic emulsifier options. One of the most commonly used synthetic emulsifiers approved for dressings are in the Polysorbate family such as the widely used polysorbate 60. Their functionalities are presented in Chap. 6 of this volume in more detail.

Dressing systems such as mayonnaise are bound by the United States standards of identity. Thus, only eggs (whole, yolks or their combinations) are the allowed as the primary emulsifying agent (Federal Register 2011). In fact, the most commonly listed emulsifying systems for retail dressing products overall is still egg yolk with a few brands still including polysorbate 60. Only ranch type dressings additionally include dairy proteins in the form of buttermilk solids as emulsifying agents since the inherent whey proteins are soluble at dressing pH's that typically range from 3.5 to 4.5. Some dressing producers use plant-based proteins such as yellow pea, soybean or canola oil seeds to provide the necessary emulsification functionality. As a frequent and allowed dressing additive, mustard flour has been shown to possess emulsification potential in terms of oil drop size reduction and emulsion stability in dressing systems such as mayonnaise as reported by Milani et al. (2013).

Meeting consumer demands with simpler, fewer and more natural ingredient names, synthetic non-ionic water soluble emulsifiers such as those in the polysorbate family are steadily becoming out of favor due to perceived health concerns. Until recently, health concerns have not been supported by any credible research showing adverse physiological effects although humans have been consuming polysorbates in a broad range of food systems for decades.

In-vivo mouse study by Chassaing et al. (2015) focused on various histological, metabolic and physiological changes or disorders after emulsifier consumption using mice models. Polysorbate 80 consumption (1% w/w in water provided ad libitum) was used to relate its ingestion with histological and physiological changes in the mouse gut. These observed changes suggested emulsifiers such as polysorbate 80 as an example of other polysorbates in general, disrupted the integrity of the gut mucosal layer. Although this study suggested polysorbates have the potential to be an irritant in the human gastrointestinal tract, additional research is needed to address some of the important gaps in this study. For example, the emulsifier daily intake was uncontrolled, the emulsifier amounts per kilogram body mass were ingested to a much higher level than those ingested by humans through dressings. Additionally, the marked known differences in GI tract physiology, volumes and gut residence times between humans and mouse requires further research to objectively define the ultimate impact of ingested polysorbate emulsifiers on human gut physiology and metabolism. While imperfect, these types of studies combined with social media amplification will likely deter growing use of synthetic emulsifiers such as the polysorbates by food producers that value and protect their brand quality.

A white paper by Andres-Brull (2016) summarizes recent efforts to meet consumer desire for natural emulsifiers. These studies evaluated the emulsification potential of various plant components such as flours from mustard, chickpea and peanut as well as fiber from potato and bamboo. Their results also highlight the challenges of identifying emulsification systems capable of substituting some of the currently used emulsifiers.

Dressing formulation strategies are also increasingly affected by global sustainability issues related to climate change and how we produce food, food ingredients and distribute food products. Animal welfare has been under public scrutiny to reform industrial farming practices toward more humane egg, meat and milk

production practices. Animal protein sources are increasingly viewed as less sustainable with excessive greenhouse gas production as well as the increase in crop acreage and water needed for livestock production rather than producing food crops directly targeted for human consumption. However, modeling by White and Hall (2017) showed that the complete elimination of animals from the human diet only partially decreased the generation of greenhouse gases generated by farm animals by growing more plants to compensate for the nutritional gap between plant and animal food sources. While their modeling predictions appear to show lesser benefits to an animal-free agriculture, it is unlikely to dampen the efforts for both large and small food companies to explore opportunities to source their ingredients including emulsifiers from non-animal sources.

This chapter will review some of the main emulsifying features of selected emulsifier systems most relevant to the current marketplace (2015–2018) in terms of their key structure function relationships. The physical properties and applications of small molecule emulsifiers such as phospholipids and polysorbates are covered in depth by Bergenståhl (2019).

9.3.1 Egg Yolk

Eggs, and in particular, the egg yolk, contain several naturally-occurring surface active components. These components are structurally and compositionally complex that include several protein species in addition to phospholipids. Moreover, the lipid components associate with certain proteins to form lipo-protein complexes. While the compositional and structural nature of these lipo-proteins components are essential for chick embryonic growth and development, these components also impart potent surface active functionality enabling the formation and stabilization of high oil phase volume emulsions with firmer and gel-like textures that are difficult for synthetic surfactants such as those in the polysorbate family to emulate.

The compositional, structural and functional characteristics of egg yolk have been researched over several decades. Briefly, egg yolks can be partitioned into the plasma and granule fractions (Anton 2007). The plasma fraction is comprised of low-density lipoproteins (LDL) while the granule fraction is primarily composed of high density lipoproteins (HDL) and phosvitin. The granule particulates deaggregate at ionic strengths and pH's relevant for mayonnaise and most other dressing systems. Anton et al. (2000) demonstrated yolk granule particulates at pH's <5 and ionic strengths >0.1 become more surface active than the native granule aggregates due to their increased capability to adsorb to larger oil droplet surface areas created by oil droplet size reduction during homogenization. As shown by Motta-Romero et al. (2017) and Laca et al. (2010), egg yolk granules offer the possibility of producing stable reduced-cholesterol mayonnaise since most of the cholesterol is associated with the LDL fraction in egg yolk plasma. Mayonnaise prepared with the egg yolk granule fraction were observed to be thicker than mayonnaises made with

whole egg yolk, but well within the textural range typical for commercial mayonnaise products.

On the other hand, emulsions prepared with egg yolk plasma behave similarly, but not identically, to those made with whole yolk indicating the primary emulsification activity can be attributed to the low density lipoprotein system according to Le Denmat et al. (2000) and Anton (2013). These low density lipoproteins have a much higher lipid content and especially enriched with phospholipids. The low density lipoproteins are surface active because of the amphipathic nature of lipo-protein complexes such that the polar protein domains are structurally distinct from the more nonpolar lipid domains consisting of phospholipids and triglycerides. The phospholipids themselves are also considered amphipathic and surface active, however, alone they are not as efficient at forming and stabilizing the rapidly forming oil droplet surfaces during the homogenization process. Phospholipids dispersed in water preferentially form micelles/liposomes which resist spreading across the air-water interfaces or oil-water interfaces. On the other hand, lipo-proteins readily adsorb to air/water and oil/water interfaces where they unfold and rapidly spread across the interface thereby delivering their bound/complexed phospholipids to the air-water or oil-water surfaces. In the case of oil-in-water emulsions, the oil droplet surfaces become populated with both adsorbed phospholipids and proteins. Egg yolk plasma lipo-protein fraction is thus well suited for creating stable oil-in-water emulsions (Anton 2013).

Dressing products and especially those using egg are temperature sensitive. Heating to pasteurization temperatures above 75 °C typical for pasteurized dairy products has catastrophic consequences on egg functionality. In particular, these high temperatures induce protein gelation in both yolk and egg white as well as the generation of undesirable aromas. However, the drivers of this temperature constraint have been addressed through a model emulsion approach with egg yolk fractions.

Le Denmat (1999) characterized mayonnaise model emulsions produced with egg yolk, granules and plasma heat treated at selected temperatures up to 76 °C. Heating egg yolk and plasma caused a greater degree of plasma protein (HDL) denaturation and reduced protein solubility compared to those in the granule protein system (HDL and Phosvitin). Egg yolk and plasma heated to 76 °C produced less stable emulsions, while emulsions produced with heat treated egg yolk granule remained as stable as those without heat treatments. These results suggest the possibility to use granules to create a more heat tolerant mayonnaise. Furthermore, in whole egg systems, heating processes to reduce microbial counts should not exceed 70–75 °C to preserved emulsification functionality.

In order to produce safe dressing products with functional egg components, egg suppliers pasteurize various egg systems such as salted whole eggs or salted egg yolks following the guidelines developed through the USDA (Froning et al. 2002). These time temperature conditions are tailored to the type of egg product in order to assure the egg products are sterile and free from pathogens such as Salmonella within the limits of detection while preserving the expected functional properties. It should be noted that final dressing products are subsequently preserved by lowering

the pH below 5 most often near pH 3.5. This is accomplished with a variety of acids such as acetic acid (vinegars), lemon/lime juice, lactic acid, citric acid, hydrochloric acid and phosphoric acid. The choice of acid depends on both the standards of identity of the particular dressing as well as taste/flavor targets.

Although hen egg compositions do not vary greatly out of shell, whole egg or egg yolk functionality can vary significantly among producers stemming from heat treatment conditions, added salt levels and mixing and freezing conditions. Furthermore subsequent egg ingredient storage conditions at dressing production sites such as temperature, number of freeze thaw cycles, and storage time can further affect final dressing properties. In addition to the above variations originating from a single egg supplier, dressing batch to batch variability can arise from the order of egg yolk addition with respect to acidulent (e.g., lemon juice, vinegar), the oil addition rate, oil temperature as well as egg yolk/whole egg ratio variations.

Although egg yolks are indeed an excellent source of emulsifier functionality, dressing producers should be setting and controlling their egg yolk specifications including egg yolk sourcing criteria, egg storage conditions and product production parameters in order to consistently attain the dressing quality standards their respective consumers expect.

9.3.2 Egg Yolk Modification

Periodic avian influenza outbreaks and the increasing shift to more humane egg production practices may lead to transient increases in egg costs. Dressing manufacturers are often under pressure to develop processes and formulations to lower the impact of rising egg costs. In addition to reducing dressing oil content, egg yolk modification to enhance its functionality remains a viable option to lower egg use levels.

Several egg treatment options have been explored to improve emulsification behavior including a range of salt, pH and enzymatic pre-treatments. Some of the more notable enzymatic treatments involve proteases and phospholipases to produce reaction products with improved emulsifying capability per gram of egg yolk solids. Enzymatic phospholipid conversion to lysophosphatides and free fatty acids has been extensively investigated using phospholipase A2 type enzymes as shown by Jin et al. (2013), Kim et al. (2009) and Daimer and Kulozik (2009). The resulting lysophosphatides are more surface active than phospholipids and have the potential to reduce egg yolk use levels. Furthermore, emulsions prepared with phospholipase A2 modified egg yolk tend to have a more gel-like textures at high oil phase volumes (>65%) typical for mayonnaise. Phospholipase D treatment of egg yolk converting phosphatidylcholine to phosphatidic acid and choline have been shown to impart improved textural heat stability characteristics of mayonnaise type dressings Buxmann et al. (2010a, b).

Enzymatic reactions inevitably increase ingredient costs because they require processing control ensuring reaction completion and total inactivation. Enzyme

sourcing requires stringent specifications to ensure proper enzymatic activity and sufficient supply to meet dressing production demands and kosher and halal requirements. While enzyme modified yolks provide sufficient stability and textural benefits with less egg solids, its ingredient label appearance as "enzyme modified egg yolk" may attract some negative attention by some consumers questioning the origin of this ingredient. Therefore careful consideration of its impact to brand image must be considered before incorporating enzyme modified egg technology to enhance product quality at lower cost.

9.3.3 Polysorbate 60

Synthetic small molecule emulsifiers such as polysorbate 60 have been used to replace the emulsification functionality lost due to lower cost formulations using less egg yolk solids. Generally the inclusion of polysorbate 60 produces emulsions with similar oil droplet size distributions to emulsions using egg yolk solids. However, all of the polysorbate emulsifiers are interfacially very potent and may not reproduce the emulsion qualities in terms of texture, mouthfeel and flavor of those produced with egg yolk.

Interfacial investigations such as those by Courthaudon et al. (1991), Mackie et al. (1993) and Cornec et al. (1998) have shown how small molecule emulsifiers promote partial displacement and rearrangement of adsorbed proteins can effect emulsion stability. It is presumed these small molecule surfactants not only rapidly adsorb to oil droplet interfaces because of their small size, they also desorb interfacial proteins by binding to their hydrophobic domains and thus creating more fluid interfaces by diminishing inter-protein interactions. More fluid oil droplet interfaces are more prone to coalescence and are more likely to reduce the intensity of droplet-droplet interactions that are important for increasing product visco-elasticity. Therefore, fully or partially replacing egg solids or other proteins with small molecule emulsifiers requires significant formulation and processing efforts to attain the desired product textural and stability characteristics.

The increasing emphasis on health and wellness issues has placed challenging demands on dressing composition. Obesity, diabetes, and cardiovascular health issues have led to an increasing desire for incorporating more natural and fewer ingredients identified by consumers as "chemical". In contrast to the singularly health focused re-formulation efforts for fat-free dressings during the 1990s, current health trends can be characterized as more complex and inter-dependent. This has been largely due to advances in medical and epidemiological studies and its rapid dissemination through various social media channels. For the food industry and in particular, the dressings and sauces area, there is pressure to reduce calories (more than just fat reduction alone), reduce sodium intake, and increasing intake of healthy oils and maintaining a more natural, if not authentic ingredient line by replacing synthetic and non-naturally occurring additives such as emulsifiers, stabilizers, colors and preservatives.

9.3.4 Plant-Based Protein Emulsifiers

Egg replacement alternatives for eggless dressing products are having an increased presence to provide animal-free ingredient options to vegan consumers as well as those committed to reducing animal sourced ingredients in their diets. Typically these egg replacement ingredients are seed proteins derived from various plant sources which can function as emulsifiers as well as texture building agents.

Dressing manufacturers have long sought plant protein sources such as soy and pea proteins to address the needs of vegan consumers. Vegetable alternatives to animal derived surface active proteins are available with a broad range of purities. Soy protein systems are well known commercially and have been incorporated into various dressing formulations for many years. Pea proteins are being increasingly used in dressings as soy alternatives due its lower allergenic incidence and lower amounts of anti-nutritive factors.

Pea and soy protein systems are capable of emulsifying oil, however they tend to be less surface active than egg yolk. One of the complexities of comparing emulsifying capabilities among various plant sources is the under-appreciated role of genotypes play within each protein source. Some of these genotypic variations may alter the seed protein amino acid sequences and thus influence their emulsification functionality. While both soy and pea protein systems are capable of producing dressing emulsions, their emulsifying capability diminishes at pH's below the pH 5 needed for dressing preservation. The comparative aspects of emulsifying activities among various soy and pea protein ingredients has been succinctly reviewed (Barać et al. 2015).

Currently, vegetable protein systems still carry beany type off-flavors originating from endogenous lipoxygenase released during the protein extraction processes. This off-flavor aspect may require additional purification efforts or flavor masking approaches to ensure broader appeal to non-vegan consumers.

9.3.5 Mustard Flour

Several egg-yolk based dressing products including mayonnaise are formulated with mustard flour. Produced primarily from the seeds of the Yellow mustard (*Sinapis alba*) plant, mustard flour has been shown to exhibit emulsion stabilizing properties in addition to providing its well-known pungent flavor attributes and associated anti-oxidant properties. Mustard flour surface-active properties derive from the seed's mucilage component as well as the seed storage proteins that are similar to other plants such as soy and canola (Patterson 2016). Comparative studies among the various commercially produced mustards have been reviewed and shown measurable differences in protein content, type and functionality (Patterson 2016). In particular, the mucilage component is particularly surface active and the yellow mustard seeds have the highest mucilage content as shown the Mustard 21 Study (2009). Milani et al. (2013) showed the yellow mustard flours produce a

measureable enhancement in emulsion viscosity and its potential to enhance emulsion performance from an ingredient that is typically included in many dressing formulations.

At low usage levels, the accompanying sulfury notes in mustard flour can complement dressing flavor/aroma, which limits the mustard flour level to maintain flavor balance due to its inherent intense pungency. Research efforts leading to the removal of mustard pungency including the inactivation of endogenous myrosinase have shown promise (Milani et al. 2013). However, none of these efforts have led to the production scale of deflavored mustard flour necessary for commercially incorporating into dressing emulsions up to 1–2% levels. It should be noted that as members of the Brassicaceae family, mustard seed contain 25–35% oil by weight and its high levels of erucic acid is a limiting factor for the use of mustard seed oil in food products. Currently, the economic argument is weak for increasing mustard seed production and limits its potential as a "naturally" occurring dressing emulsifier at higher usage levels.

9.4 Hydrophobic Starch

Reduced-oil dressing formulations often include starches as texturants to make up for the textural and mouthfeel functionalities lost upon oil phase volume reduction. Alone, starches are not considered emulsifiers and do not exhibit surface activity. However, there has been an enlarging body of research centered on hydrophobic starch systems to understand the modification chemistry and subsequent effects on product properties. Although hydrophobic starch systems are not naturally occurring and thus labeled as a "modified food starch", it may not face the same consumer hurdles for acceptance as other synthetic emulsifiers. Consumers have grown accustomed over the last few decades with having "modified starch" in various food products with its relatively innocuous sounding name. As a result, hydrophobic starches are likely to experience continued development and usage in food products such as dressings.

Hydrophobic starch surface activity originates from modifying starch with octenyl succinic anhydride (OSA) which esterifies the hydrophobic octenyl moiety to the starch polymer glucose moieties. These hydrophobic starches readily stabilize the oil-water interfaces of oil droplets as demonstrated by Nilsson and Bergenståhl (2006). Their studies also showed OSA starches rapidly adsorb to oil-water interfaces during emulsification with surface coverages ranging from 4 to 16 mg/m^2 depending on the starch source. As a kinetically driven process, OSA starches rapidly adsorb to an oil droplet surface. This rapid adsorption behavior leads to denser packing at the interface. The adsorbed starch is slow to unfold produce oil droplets stabilized through steric forces. These observations are consistent with the ability of adsorbed hydrophobic starches to impart oil droplet stability through steric forces that maintain sufficient distances among oil droplets thus preventing or certainly slowing oil droplet coalescence rates.

Several major food starch suppliers offer an array of OSA modified starch products. Depending on the starch plant source, whether it's modified prior or after gelatinization or after mild hydrolysis, will affect how it performs as an emulsifier/texturizer. Bao et al. (2003) showed the OSA reaction can be applied to a broad range of starch sources as well as starch hydrolysates as demonstrated in Billmers and Mackewicz (1995). Due to the decreasing solubility of the alkyl succinic anhydrides as the alkyl carbon number increases beyond C_8, the commercially available hydrophobic starches have been limited to the OSA. More intense hydrophobicity is expected with longer alkyl residues, however reaction conditions need to be further explored to increase reactant solubility in order to increase reaction yield.

In a study comparing two commercially available OSA modified waxy maize starches, Dokic et al. (2008) showed the OSA starch solutions with larger intrinsic viscosities were more impactful than just its ability to reduce oil droplet size for increasing emulsion visco-elasticity and slowing creaming rates. This study demonstrated effect of OSA-starch in dressing type oil-in-water emulsions is dominated by its inherent solution conformation as well as its adsorbed state on oil droplets.

Although commercially produced from several starch sources, OSA-starch systems can still benefit from further research and development exploiting both the conformational and emulsification functionalities leading to even more functional options for dressing emulsion producers.

9.5 Concluding Remarks

Dressings with egg-based emulsifiers have become the gold standard with respect to product texture, flavor and stability. However, the evolution of dressing emulsifier systems has trended toward naturally occurring surface active systems and especially over the last 5 years. The most prominent emulsifier system continues to be egg yolk and its various modifications. In parallel, there has been a steadily increasing exploitation of plant proteins from soy, canola and yellow pea as dressing emulsifiers. Compared to egg yolk systems, plant based proteins are less surface active and often possess off flavors thus requiring additional processing/conditioning steps to create emulsions closer to parity of those created with egg. Research efforts exploiting natural emulsifier sources such as those described by Andres-Brull (2016), pave the way for the development and refinement of emulsification functionality. Recalling that egg yolk functionality is dependent on endogenous lipoprotein complexes, creating lipo-protein complexes with selected plant protein systems may be a promising option.

While the consumer desire for "cleaner" ingredient labels may appear to limit the range of protein modification, the expansion of hydrophobic starch varieties promises to deliver emulsions with both enhanced stability with desired textural properties in spite of being classified as "modified starch". Using egg yolk modification as a precedent, efforts to enhance plant protein emulsifying properties through various enzymatic and non-enzymatic routes should be pursued and encouraged. Given the

high level of interest to develop plant proteins as egg yolk alternatives coupled with advances in crop genomics, plant protein emulsifier development should continue to grow in parallel with those developing hydrophobic starches.

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Chapter 10
Applications of Emulsifiers in Baked Foods

Frank Orthoefer and Dennis Kim

10.1 Introduction

Emulsifiers are multifunctional ingredients when used in bakery products. The three major functions are (1) to assist in blending and emulsifications of ingredients, (2) enhance the properties of the shortening, and (3) beneficially interact with the components of the flour and other ingredients in the mix. Some of the specific functions are uniquely described as creaming, dough conditioning and crumb softening. This chapter discusses the activity and functional role of emulsifiers in baked products.

10.2 History of Bakery Emulsifiers

The development of emulsifiers for bakery products parallels the development of emulsifiers for shortenings. The term "shortening" was initially used to refer to the fats used to "shorten" or tenderize baked foods. The composition of the shortening has progressed from natural fats to blends of oils, hydrogenated fats and hard fats as well as trait modified oils, used in food preparation. Shortenings intended for bakery products, however, may include additives such as emulsifiers, antioxidants, antifoam and metal scavengers. Bakery shortenings may be the tenderizer as well as the ingredient that affect structure, stability, flavor, storage stability, eating characteristics and eye appeal. Many of the functional effects are due to, or are enhanced by, the emulsifier added as a component of the shortening.

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Historically, animal fats were used for bakery products because of their natural plasticity and flavor (O'Brien 1996). Lard was the preferred animal fat because of its pleasing flavor. With the excess of cottonseeds and cottonseed oil in the market, vegetable shortenings were developed by the cottonseed industry early in the twentieth century. Initially, cottonseed oil was blended with lard as a "lard compound" or simply "compound shortening." Hydrogenation was invented in 1910. This allowed the production of vegetable based substitutes for semi solid (plastic) animal fats and permitted the development of products with improved functional properties.

Along with a process to modify the melting properties of fats or oils (hydrogenation) improved methods for processing the oil including refining, bleaching and deodorization. The fully processed products possessed improved oxidative stability, uniformity and enhanced performance. Knowledge of lipid chemistry led to improvements in alcoholysis, esterification, interesterification and isomerization. These advances in lipid chemistry led to new emulsifiers and improved shortening formulations. High ratio shortening was introduced around 1933. These shortenings contained mono- and diglycerides. The emulsifiers produced finer dispersions of fat particles in the dough giving strengthened cake batters. Stronger cake batters permitted increased water and sugar addition resulting in sweeter tasting, more tender cakes. The high-ratio shortenings possessed excellent creaming properties. Moist, high volume, fine-grained, even-textured cakes were produced. Icings were also improved (Hartnett 1977).

Emulsifier development also advanced in the 1930s (Stauffer 1996a). Specialty shortenings were formulated. Commercial layer cakes, pound cakes, cake mixes, cream fillings, icing, whipped toppings, bread and sweet dough shortenings were created. This development of specialty emulsifiers resulted in improvements in processing and improved product performance for the retail, food service and food processing industries. In addition to the traditional plastic shortenings, liquid shortenings, fluid shortenings and powdered products were produced (O'Brien 1995). All these products involved formulations with emulsifiers.

10.3 Definition of Emulsifiers

Emulsifiers are surface active agents that promote the formation and stabilization of an emulsion. A surfactant is also a surface active agent. The terms emulsifiers and emulsifying agent, surfactant and surface active agent are synonymous and used interchangeably in the literature. The terms "emulsifier" and "emulsifying agents" are, strictly speaking, chemical compounds capable of promoting emulsions or stabilization of emulsions by their effect on interfacial tension. Surfactants for foods may include not only emulsifiers but also compounds with other functions such as protein or starch interaction.

The roles of emulsifiers and of the shortening are intimately bound in bakery products. Generally, the food emulsifiers for bakery products supplement and improve the functionality of a properly developed shortening. Emulsifiers act as

Table 10.1 Emulsifiers used in shortenings

Mono- and diglycerides	Sorbitan monostearate
Lecithin	Polysorbate 60
Lactylated monoglycerides	Polyglycerol esters
Calcium stearoyl lactylate	Succinylated monoglycerides
Sodium stearoyl lactylate	Sodium stearoyl fumarate
Propylene glycol monoesters	Sucrose esters
Diacetyl tartaric esters of monoglycerides	Stearoyl lactylate
Ethoxylated monoglycerides	–

Table 10.2 Examples of nonemulsified and emulsified shortenings

Non-emulsified	Emulsified
All-purpose	Cake and icing
Puff pastry	Household
Pie crust	Filling
Cookie	Cake mix
Danish roll-in	Yeast raised
Donut fry	Specialty cake

lubricants, emulsify oil or fat in batters, build structure, aerate, improve eating quality, extent shelf life, modify crystallization, prevent sticking and retain moisture. A list of emulsifiers used in shortening is given in Table 10.1. The selection and addition of an emulsifier to a shortening base may significantly change the application of the shortening (Table 10.2).

10.4 Emulsifier Function in Baked Goods

Baked goods without emulsifiers have been described as tough, dry, stale, leathery, or tasteless (Brandt 1996). Current processing, distribution and storage of baked goods require the use of additives that maintain quality and freshness. Fewer bakeries, longer distribution and extra time before consumption require longer shelf life of finished baked goods.

Emulsifiers are commonly used in many food products. These supplementary materials or food additives are used to:

1. Compensate for variations in raw materials
2. Guarantee constant quality
3. Produce alternative products
4. Preserve freshness and eating properties
5. Facilitate processing (Schuster and Adams 1984).

Emulsifiers promote the emulsification of oil in water. This is true for baking emulsifiers; however, emulsification is often of secondary importance in baked goods. Starch complexing, protein strengthening, and aeration may be the primary function. Fat sparing effects are also of importance.

Table 10.3 Wheat flour composition (dry basis)

Wheat flour	Percent
Starch	70.0–75.0
Protein	11.5–12.5
Pentosanes	2.0–2.5
Lipid	1.0–1.5
Crude fiber	0.2
Ash	0.5

Wheat flour contains numerous components (Table 10.3) that may interact with emulsifiers. Starch is the major flour component followed by protein. The interactions between protein, carbohydrates and lipids is significant for processing of wheat flour and flour/water doughs. The interactions between emulsifiers and flour components are multifaceted and account for the improved functionality and performance of baked products.

The use of surfactants in bakery products is regulated in most countries. The European Economic Community (EEC) number and US FDA Code of Federal Regulations (21 CFR) for the most common food emulsifiers are shown in Table 10.4. The specification and assay procedures for all emulsifiers are published in the Food Chemical Codex (2004).

Bakery products are the largest users of food emulsifiers (Stauffer 1996b). Yeast raised and chemically leavened products are the most important segments. Food emulsifiers are also included in cookies, crackers, pasta and snacks. Recent figures indicate about 400–500 million pounds of emulsifiers are used in the US, food industry with a market value of about $500 million. The baking industry accounts for about 50% of the total food emulsifiers market (Brandt 1996). Annual growth in the production of food emulsifiers is estimated at about 2.0–3.0%.

10.5 Role of the Shortening

The shortening when mixed into a hydrated dough or batter interrupts the development of the gluten network. Literally, the structure is “shortened” and the baked product is tender. The shortening also contributes to the quality of the finished product by imparting a creamy texture and rich flavor, tenderness and uniform aeration for moisture retention and size expansion. The oil or fat based ingredients are formulated and processed to a plasticity that allows spreadability and dispersion thoroughly and uniformly in a dough, icing or batter over a wide temperature range. The ability of the fat to disperse in streaks or films helps to lubricate the structure of the dough during mixing. The fat dispersion prevents the starch and protein in the flour from compacting into dough mass (Stauffer 1996b).

The characteristics of the fat that are important for shortening formulations include melting point, oxidative stability, solid fat index and plasticity. Plasticity is used to define the characteristics of the shortening that are most important to its functionality (Erickson and Erickson 1995).

Table 10.4 CFR (US FDA) and EEC number of approved food emulsifiers

Emulsifier	U.S. FDA (21 CFR)	EEC number
Mono-glycerides and diglycerides (GRAS)	184.1505	E 471
Succinyl monoglyceride	172.830	–
Lactylated monoglyceride	172.852	E 472
Acetylated monoglyceride	172.828	E 472
Monoglyceride citrate	172.832	E 472
Monoglyceride phosphate (GRAS)	184.1521	–
Stearyl monoglyceride citrate	172.755	E 471
Diacetyl-tartrate ester of monoglyceride (GRAS)—DATEM?	184.1101	E 472
Polyoxyethylene monoglyceride	172.834	–
Propylene glycol monoester	172.854	E 477
Lactylated propylene glycol monoester	172.850	–
Sorbitan monostearate	172.842	E 491
Polysorbate 60	172.836	E 435
Polysorbate 65	172.836	E 436
Polysorbate 80	172.840	E 433
Calcium stearoyl lactylate	172.844	E 482
Sodium stearoyl lactylate	172.846	E 481
Stearoyl lactylic acid	172.848	–
Stearoyl tartrate	–	E 483
Sodium stearoyl fumarate	172.826	–
Sodium lauryl sulfate	172.822	–
Dioctyl sodium sulfosuccinate	−172.810	–
Polyglycerol esters	172.854	E 475
Sucrose esters	172.859	E 173
Sucrose glycerides	–	E 474
Lecithin (GRAS)	184.1400	E 322
Hydroxylated lecithin	172.814	E 322
Triethyl citrate (GRAS)	184.1911	–

Shortenings are processed to various plasticity ranges (Weiss 1983; O'Brien 1995). Narrow plastic range ingredients have a steep solids profile and melt rapidly. These ingredients are commonly used in cream icing products or as a filler fat for hard cookies where melting near body temperature is required. Wide plastic range shortenings contain 15–30% solids over a broad temperature range and resist breakdown during creaming. Their plastic nature enables them to spread readily and combine thoroughly with the other solids or liquids without breaking or having liquid oil separating from the crystalline fat. Commercial shortenings are prepared by carefully cooling, plasticizing and tempering of correctly formulated blends of melted fats and oils. The plasticizing process is often referred to as "Votation."

The size of the fat crystals in a plasticized shortening has a major influence on the rheological properties of the shortening. A small crystal size with a large surface

area is required to bind the liquid oil in the shortening. Typical crystal sizes are from 5 to 9 μm (Chawla and deMan 1990). Crystal size is controlled by the source of the hard fat used (O'Brien 1996). The smaller crystalline form is referred to as β′ and the larger form is β. Plastic shortenings in the β′ configuration consist of small, uniform needle-like crystals with a smooth texture. These aerate well and have excellent creaming properties.

Two major sources of β′ crystalline fats are often used in formulation of votated shortenings. These are cottonseed and palm oil, often, fully hydrogenated to less than I.V. (iodine value) of 10. The use level varies from 8% to 15% of the final shortening formula.

10.6 Role of the Emulsifier

Addition of emulsifier to the shortening promotes the emulsification of the shortening in the dough or batter. Much of the development of shortenings has concentrated on the addition of the emulsifier or emulsifier system to an all-purpose shortening base although specialty liquid, narrow plastic range and special purpose emulsified products have been produced (O'Brien 1995). Today, because of the focus on trans fatty acid free ingredients, much interest has focused on emulsifier systems that permit the use of non-hydrogenated, trait modified oils as the shortening.

The general benefits of including emulsifiers in shortenings are:

1. Increased shelf life.
2. Improved tenderness and flavor release.
3. Reduced mixing time and mixing tolerance.
4. Improved machinability.
5. Better water absorption.
6. Improved volume.
7. Improved hydration rate of flour and other ingredients.
8. Better texture and symmetry.
9. Reduced egg and shortening usage.

10.6.1 Monoglycerides and Derivatives in Bakery

The monoglycerides in their many forms are the most used emulsifier in bakery products. Seldom is an ingredient label found that does not list this type of emulsifier. The preparation of monoglycerides begins with reacting glycerin with edible fats and oils or fatty acids in the presence of a catalyst (Henery 1995). The important characteristics are melting point and monoglyceride content. Commercially available products vary from 40% to 95% monoglyceride content. Two crystalline forms are generally present: alpha and beta. The alpha form is the most functional in bakery products. The major

variables involved in the production of monoglycerides are source of the fat, monoglyceride content, iodine value or degree of unsaturation, and fatty acid composition. Approximately 300 million pounds of monoglycerides are used in the United States in yeast-raised bakery products (Knightly 1988). An equal amount was believed to be used in cakes, icings, and other applications. Cakes prepared with shortenings containing monoglycerides have improved aeration and sugar holding capacity. Breads possess an improved shelf life due to retarded staling rate. Various techniques have been used to improve monoglycerides through chemical modification or formulation with additional emulsifiers. The form of monoglycerides marketed for bakery applications include plastic, hydrated, powdered and distilled monoglycerides.

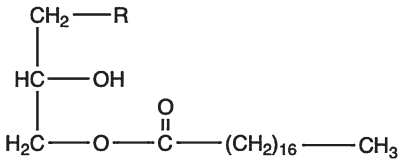
In addition to their anti-staling benefit, monoglycerides in bakery products results in:

- Reduction of interfacial tension.
- Improved dispersion of ingredients.
- Increased aeration.
- Greater foam stability.
- Modification of fat crystal.

Several derivatives of monoglycerides have been prepared (Fig. 10.1). Two main functional types are generally found in bakery applications: dough strengtheners and alpha-tending monoglycerides. The "dough strengtheners" include succinylated monoglycerides (SMG), ethoxylated monoglycerides (EMG), and diacetyl tartaric acid esters of monoglycerides (DATEM). They are also used as emulsifiers, starch and protein complexing agents and foam stabilizers. The alpha-tending emulsifiers include GMS (glycerol monostearate), LacMG (lactylated monoglycerides), AcMG (acetylated monoglycerides) and PGME (propylene glycol monoesters). The alpha-tending emulsifiers, normally used in cake mix production, contribute to the emulsification of the shortening in the water phase of the batter as well as incorporating air into the fat phase. The alpha-tending monoglycerides are believed to form a film at the oil/water interface resulting in a stable emulsion that prevents the liquid oil present in the shortening from interfering with aeration during cake batter mixing.

10.6.2 Sorbitan Emulsifiers

Sorbitan monostearate is a commonly used oil soluble, low HLB nonionic emulsifier. Reaction of the sorbitan esters with ethylene oxide results in the formation of the polyoxyethylene sorbitan monostearate or polysorbate emulsifiers (PS60 or polysorbate 60) (Fig. 10.2). Sorbitan esters are excellent emulsifiers for improving aeration, gloss and stability of icings and coatings. They generally function as emulsifiers, aeration agents, and lubricants in cakes, toppings, cookies and crackers. Polysorbate 60 is often used as a dough strengthener at about 0.2% of flour weight. Polysorbate 60 is also used in combination with glycerol monostearate and propylene glycol monostearate in fluid cake shortenings.

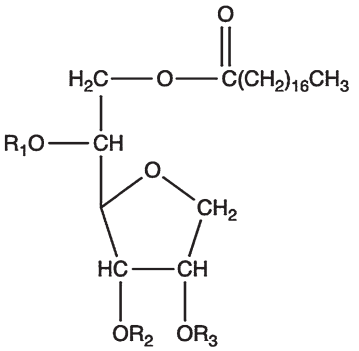


When R Equals	Emulsifier
HO——	Glycerol monosterate (GMS)
——$OOCH_2CH_2COO$——	Succinyl monoglyceride (SMG)
CH_3CH(OH)—COO——	Lactylated monoglyceride (LacGM)
H_3C—COO——	Acetylate monogyceride (AcMG)
——OOC—CH(OOCCH)—CH(OOCH$_3$)—COO——	Diacetyl-tartaric acid ester of monoglyceride (DATEM)
$H(CH_2CH_2O)_nO$——	Polyoxyethylene monoglyceride or ethyoxylated monoglyceride (EMG)
H——	Propylene glycol monoester (PGME)

Fig. 10.1 Monoglycerides and derivatives

10.6.3 Anionic Emulsifiers

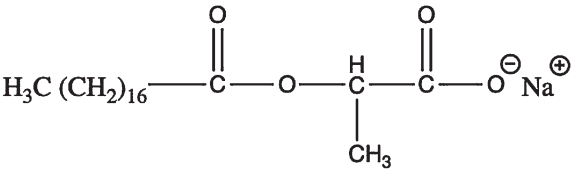
The anionic emulsifiers include SMG, DATEM and other lactic acid derivatives (Fig. 10.3). Sodium stearoyl lactylate (SSL) and the calcium form (CSL) are widely used. Both are employed as dough strengtheners.



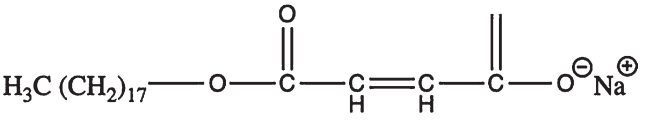
Where

$R_1, R_2, R_3 = H$	Sorbitan monostearate
$R_1 = H\ (CH_2CH_2O)_n$ —— $R_2 = H$ $R_3 = H$	Polyoxyethylene (20) sorbitan monostearate (Polysorbate 60)
$R_1 = H\ (CH_2CH_2O)_n$ —— R_2 and $R_3 = H_3C\ (CH_2)_{16}CO$ ——	Polyoxyethylene (20) sorbitan tristearate (Polysorbate 65)

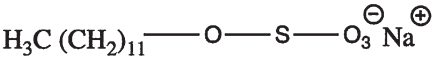
Fig. 10.2 Sorbitan esters and derivatives



Sodium Stearyl lactylate (SSL)
(also in calcium form)



Sodium stearyl fumarate (SSF)



Sodium lauryl sulfate (SDS)

Fig. 10.3 Anionic surfactants

SSL may be added as a stabilizer to the hydrated monoglycerides preparations. The lactic acid emulsifiers also act as anti-staling, aeration aids and starch/protein complexing agents.

10.6.4 Polyhydric Emulsifiers

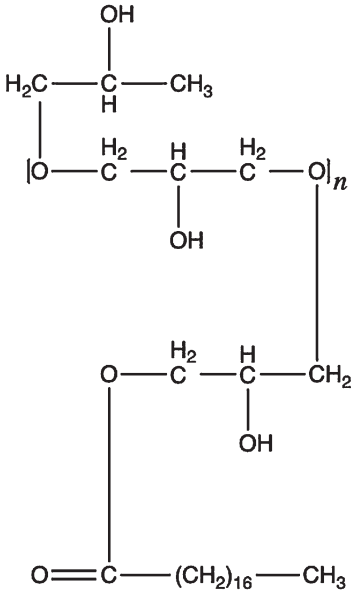
The main polyhydric emulsifiers are the polyglycerol esters and sucrose esters (Fig. 10.4). Both have multiple applications as emulsifiers for foods and bakery products, particularly the sucrose esters. They provide emulsifying, stabilizing and conditioning properties in baked goods. A maximum of eight hydroxyl groups in sucrose may be esterified. The degree of esterification affects the hydrophilic-lipophilic balance (HLB) of the sucrose ester (Table 10.5). Sucrose esters are used as a noncaloric fat substitute when six or more of the hydroxyls are esterified. Deep fried snacks have reduced caloric content due to noncaloric sucrose esters of the frying oil substitute.

10.6.5 Lecithin

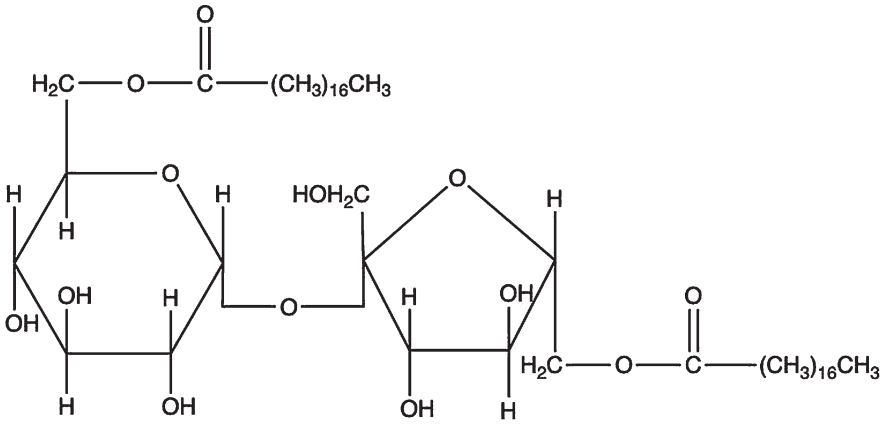
Commercial lecithin is a co-product of soybean oil production. Limited quantities are produced also from corn oil and sunflower oil. Lecithin is obtained by water washing of the filtered crude soybean oil. The hydrated lecithin is easily separated from the oil and is vacuum dried. Crude lecithin is a dark colored, viscous mixture composed mainly of a mixture of phospholipids (Fig. 10.5). Triglycerides, to copherols, sterols and glycolipids are present. Various purified grades of lecithin are produced by bleaching and fractionation as well as by chemical modification (Schmidt and Orthoefer 1985). Commercial lecithin products are specified based on the acetone insoluble fraction (a measure of the phospholipid content), viscosity and color. Lecithin is also found in egg yolk, butter, beans and nutmeats. Lecithin is usually an inexpensive emulsifier used for anti-stick properties as well as emulsification and controlled wetting of dry mixes.

10.7 Emulsifier Interaction with Bakery Components

Emulsification and lubrication (shortening) by the emulsifier accounts only partially for the beneficial effects observed when they are added to baked products. Proteins and lipids contribute to the functional properties of the flour. Emulsifiers interact with various flour components especially the starch, protein and lipids, as well as the added ingredients.



Polyglycerol monostearate



Sucrose diester

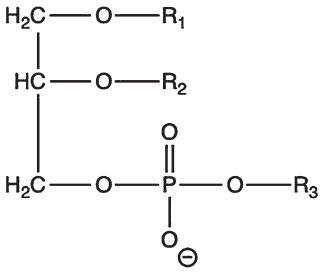
Fig. 10.4 Polyglycerol and sucrose esters

Table 10.5 Sucrose ester surfactants

Percent monoester	Percent diester	Percent triester	Percent tetraester	HLB
71	24	5	0	15
61	30	8	1	13
50	36	12	2	11
46	39	13	2	9.5
42	42	14	2	8
33	49	16	2	6

After Stauffer (1996c)

Fig. 10.5 Lecithin



(R_1 and R_2 are fatty acids)

If R_2 = Choline ethanolamine inositol serine

Then Phosphatidylcholine

Phosphatidylethanolamine

Phosphatidyl inositol

Phosphatidylserine

10.7.1 Starch

Starch exists in a helical, coiled structure with six glucose residues per turn of the helix. This structure is a hollow cylinder with a hydrophilic outer surface and a hydrophobic inner core. The inner space is about 45 nm in diameter. Straight-chain alkyl molecules such as palmitic or stearic acid will fit in the inner space. The n-alkyl portion of emulsifiers such as present in GMS form a complex with the helical regions of the starch. It is this complex that retards starch crystallization, often called "retrogradation," slowing the staling process.

Emulsifiers affect the cooking and swelling properties of starch (gelatinization). This may be due to the rate of gelatinization, gelatinization temperature, peak viscosity or gel strength. Trials with starch pastes containing monoglycerides showed that maximum complexation occurs with monopalmintin (Lagendijk and Pennings 1970). Longer and shorter chain saturated fatty acid monoglycerides reacted to a

lesser extent. Unsaturated fatty acid monoglycerides react to a lesser extent due to the bend in the fatty acid chain at the unsaturated bond (Hahn and Hood 1987).

Other surfactants also modify the gelatinization of starch. DATEM is generally found to be less interactive than GMS or SSL. GMS raises the swelling temperature and results in increased paste viscosity. SSL also increases paste viscosity (Schuster and Adams 1984). Overall the interaction between emulsifier and starch takes place at the surface of the starch granule and the starch/surfactant complex stabilizes the granule, retarding water penetration and swelling as the temperature is increased (Lakshmi Narayan et al. 2006).

During bread making, only small amounts of emulsifiers are bound to starch in the sponge stage and during mixing. Binding does not occur until the temperature is increased to near the gelatinization temperature. The formation of the starch complex is principally with the amylose or linear starch fraction. Both the degree of interaction and solubility of the complexes are dependent upon the type of emulsifier.

10.7.2 Protein

The wheat flour proteins, gliadin and glutenin, form a viscous, colloidal complex known as "gluten" when mixed into dough. Lipids are involved in the formation of the gluten complex. The properties of gluten are dependent upon the lipids and emulsifiers present. Lipophilic portions of surfactants interact with hydrophobic regions of proteins contributing to unfolding or denaturation of the protein. Generally, surfactants contribute to protein denaturation, enhancing interfacial adsorption and emulsion stabilization. The desired result of the protein interaction with emulsifiers is called dough strengthening.

Most commercial dough strengtheners are anionic surfactants. The association of the lipophilic portion of the emulsifier with the hydrophobic area of the protein incorporates the negative charge into the complex with subsequent aggregation in the dough. The overall effect is aggregation of the gluten protein and an increase in dough strength.

The ionic surfactants induce protein insolubilization resulting in increased viscosity and elasticity of the dough. Nonionic surfactants disrupt the hydrophobic portion of the protein leading to reduced dough viscosity and elasticity and increased protein extractability. A blend of emulsifiers generally shows the best dispersability and functionality.

10.7.3 Lipids

Wheat flour contains 1.4–2.0% lipids divided into free (0.8–1.0%) and bound (0.6–1.0%) forms. They may be further divided into nonpolar (50.9%) and polar (49.1%) forms. The bound lipids exist as starch inclusion complexes. The non-starch free lipids, about 85% of the total, participate in the chemical, physical and

biochemical processes important for the preparation of baked goods. The nonstarch lipids consist of glycolipids, phospholipids and stearoyl esters. Interaction between nonstarch lipids and emulsifiers is limited.

Nonpolar lipid addition to untreated flour results in deterioration of baking properties (Schuster and Adams 1984). Addition of polar lipids to untreated flour increases loaf volume in bread making. The improvement is likely based on the effect of galactolipids and phospholipids. Emulsifiers may interact with the water phase of the dough, forming associated lipid-water structures with free polar flour lipids (Krog 1981). Emulsifiers may compete with the naturally occurring lipids in wheat flour for the reactive groups of the wheat flour dough. Their effect on protein components was reduced as well.

10.8 Applications in Baked Goods

10.8.1 Yeast-Raised Products

The function of emulsifiers in yeast-raised products includes dough conditioning, strengthening and crumb-softening. The direct and indirect action of the emulsifier begins with dough preparation and ends with oven baking and storage (Fig. 10.6). The first stage begins with wetting and dispersing activity then follows with interaction with flour components during mixing and in the baking process itself.

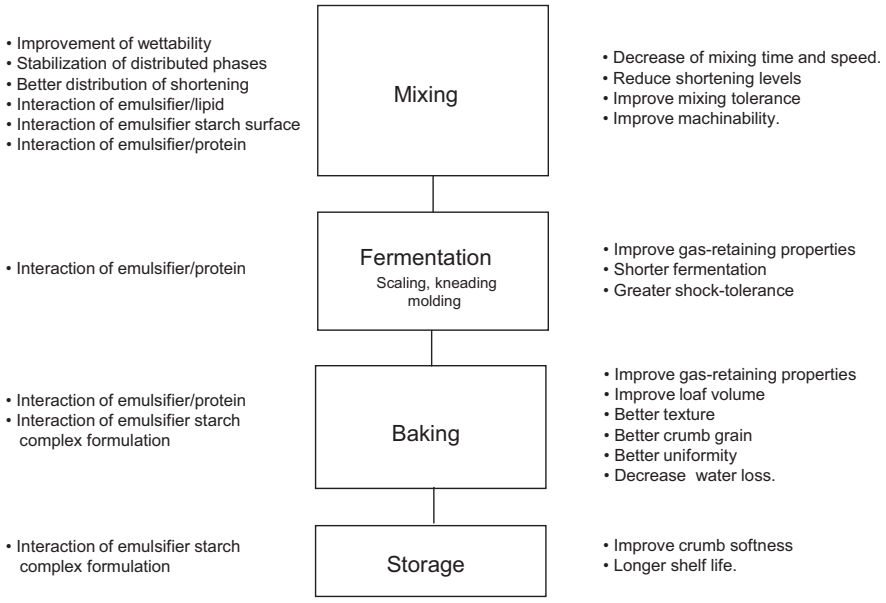


Fig. 10.6 Influence of emulsifiers on production and quality of baked products. (Adapted from Schuster and Adams 1984)

10.8.1.1 Dough Conditioning

Dough conditioning refers to the development of less tacky, more extensible doughs. They may be processed through machinery without tearing or sticking. These doughs result in a product of finer crumb structure, improved volume and symmetry. These characteristics include:

- Increased mixing and machining tolerance of the dough.
- Increased tolerance to variation in ingredients.
- Diminished knockdown during handling.
- Assist in maximum dough absorption.
- Reduced shortening requirements.
- Improved loaf volume, structure, texture and other quality attributes.
- Extended keeping quality.
- Facilitates variety bread production.

In the production of yeast-raised products, the mixing of the dough results in gluten-gluten bonding through disulfide linkages. Development of the linkage is often incomplete resulting in weak dough structure. The gas produced by the yeast escapes through the weak portion of the gluten films. Gas cells having weak gluten cell walls have a tendency to collapse.

Dough strengthening emulsifiers increase the degree of gluten-gluten binding sites and/or bridges that supplement disulfide linkages. This results in stronger gluten films. The benefits from the dough conditioners are:

- Improved tolerance to variation in flour quality.
- Drier doughs with greater resistance to abuse.
- Improved gas retention giving lower yeast requirement, shorter proof times and greater finished product volumes
- Uniform internal grain, stronger side walls and reduction of "cripples."
- Reduced shortening requirements without loss of volume, tenderness or slicing ease.

The highly functional dough strengtheners are calcium stearoyl lactylate (CSL), ethoxylated monoglycerides (EOM), polyoxyethylene sorbitan monostearate (PS-60), succinylated monoglycerides (SMG) and sodium stearoyl lactylate (SSL) (Tenney 1978). Loaf volumes for the various conditioners were compared for fully proofed dough shocked to mimic abuse in production. An increase in loaf volume of about 10% was found for CSL and SSL, with EOM and PS-60 giving slightly less increase, and SMG giving only about 5% increase in loaf volume.

10.8.1.2 Crumb Softening

Emulsifiers that complex with starch are referred to as "crumb softeners." The mechanism of activity is the result of an amylose complex being formed. The staling of bread is also believed to result from amylose crystallization. During bread

preparation and baking, amylose polymers associate upon cooling forming a rigid gel after 10–12 h. After baking, amylopectin, the branched chain starch fraction, crystallizes more slowly resulting in firming of the bread in 3–6 days. When crumb softeners are added, less free amylose occurs and therefore less is available to form a rigid gel. The emulsifier softens the initial crumb. No change occurs with the amylopectin fraction. It gradually crystallizes to a firmer texture whether or not treated with crumb softeners.

The effects of various crumb softeners as measured by compressibility after 96 h of storage were compared by Tenney (1978). The most effective softeners were the lactylates and SMG. Plastic mono- and diglycerides and hydrated distilled monoglycerides were also effective. The polysorbate 60, EOM and lecithin had little starch complexing activity. The lactylates and SMG act as both conditioners and crumb softeners.

The use level of crumb softeners varies. The most commonly used crumb softeners are the water emulsions or hydrates, of mono-diglycerides. The hydrates contain 22–25% solids and are used from 0.5% to 1% flour weight. The hydrates are significantly more functional than the nonhydrated forms. Water dispersible blends of distilled monoglycerides are also utilized. These blends contain unsaturated monoglycerides to promote rapid hydration in the sponge, brew or dough stage.

10.8.1.3 Emulsifier Blends

Lecithin has been used in breads and baked goods longer than any other emulsifier. Lecithin gives higher ductility through interaction with the gluten. Other activity claimed for lecithin is delayed staling and reduction of shortening. A synergistic effect also occurs between lecithin and monoglycerides. The monoglyceride-lecithin blends produce a better crumb grain, softer bread and higher loaf volumes. Ethoxylated monoglycerides combined with monoglycerides is also an effective dough conditioner. The negative effects of liquid oils in place of “solid” shortenings in bread production are overcome with this combination.

DATEM also acts as a dough conditioner, spares shortening and is an antistaling agent in combination with glycerol monostearate. Others include SMG, sucrose esters, polysorbate 60, SSL and CSL. The SSL and CSL can form complexes with gluten acting as a dough strengthener.

10.8.2 Chemically Leavened Products

10.8.2.1 Cakes

The role of the emulsifier in layer cakes or snack cakes is diverse and includes aeration, emulsification and crumb softening. The aerated structure of batters depends on whipped-in-air and gas (CO_2) from the leavening agent. The emulsifier lowers the surface tension of the aqueous phase improving the amount of air that can be whipped into the batter. Large amounts of finely divided air cells are important for the

development of uniform grain (Handlemann et al. 1961). The dissolved CO_2 evolves at air cell sites and does not spontaneously form bubbles. If the original batter contains many small air cells, the final cake will have a larger volume and fine (close) grain. The creaming of the sugar and shortening has a major influence on air incorporation. The incorporation of monoglycerides in the plastic shortening (3–5% alpha-monoglycerides) ensures numerous small air cells being created during beating or creaming.

Cake batter is an aerated emulsion. The integrity of the air cells determines cake volume and uniformity. Shortening is antifoam that disrupts foam cells. Emulsifiers, however, coat the exterior of the fat particles, protecting the integrity of the air cell (Wooten et al. 1967). Use of appropriate emulsifiers has permitted the use of liquid oils where only solid shortening could previously be used.

Light, tender, moist cakes are preferred by the consumer. Emulsifiers provide the desired aeration, emulsification and crumb softening. Crumb softening in cakes is a function of moisture retention, shortening activity and starch complexing. It is the same as for breads. The emulsifier complex with the starch softens the product.

Several types of emulsifiers are used in cakes. Propylene glycol monoester (PGME) is used at 10–15% of the shortening. Monoglycerides and mixtures of lactated monoglycerides with PGME are also used in cake mixes.

In baker's cakes, emulsifier selection depends on formula, production equipment and labeling requirements. Using soybean oil as the shortening, a hydrated blend of emulsifiers such as PS-60, SSL, sorbitan monostearate and distilled monoglycerides works well. Fluid shortenings are produced containing lactated monoglycerides. The traditional baker's cake system is a plastic shortening with 5–10% monoglycerides (4% alpha-monoglyceride content). Packaged cake mixes often use emulsified PGME at 10–15% of the oil. The cakes are unusually tender and are not suited to commercial cake production.

Emulsified cake shortenings are also used for cake donuts. The amount of air entrapped during creaming determines the grain in the final donut.

10.8.2.2 Cookies and Crackers

Emulsifier use in cookies and crackers is limited. However, they do play a role in controlling spread during baking, improve cutting and appearance, and improve texture.

Certain emulsifiers control spread of the cookie dough during baking (Table 10.6). This likely occurs because of modification of the viscosity of the dough. Cookie dough with SSL shows increased spread compared to a nonemulsified control (Rusch 1981). The SSL may interact with the starch granule delaying hydration of the granule and subsequent gelatinization (Tsen et al. 1973).

Lecithin that is highly fluidized with other oils or fatty acids is widely used as a release agent in cookie baking for release from rotary dies. Heat-resistant lecithins, such as those modified with acetic anhydride are especially adaptable to this application. Lecithin is used in cookie and cracker formulations at 0.25–1.0% of flour weight. It may be added with the shortening at the creaming stage or simply combined with the shortening when votated.

Table 10.6 Spread ratios of cookie doughs with different emulsifiers (data from Tsen et al. 1973)

0.5% Additive	Spread ratio
Monoglyceride	8.3
Ethoxylated monoglyceride	8.8
Sodium stearoyl fumarate	10.4
Sodium stearoyl lactylate	10.0
Sucrose monopalmitate	9.8
Sucrose mono- and distearate	9.6
Sucrose distearate	9.7
Sorbitan monostearate	9.2
Polysorbate 60	9.3
Succinylated monoglycerides	9.2

Antistaling is of less significance in cookies and crackers since they are of lower moisture content. The greasiness of high shortening levels is reduced by the addition of small amounts of lecithin. Lecithin in general produces a "drier" dough with equivalent moisture and shortening levels. The drier dough is more machinable. Other benefits attributed to lecithin are reduced mixing times and dough development with more tender cookies.

SSL is also promoted for cookies and cracker improvement. When incorporated into the dough at 0.25%, flour basis, the SSL produces a finer grained, more uniform pattern of surface cracks. The resistance to shear (firmness) decreases improving eating quality and permits reduction in shortening (Tenney 1978). Levels are 0.25% SSL in cookies and 0.1% in crackers based on flour weight.

10.8.3 Extruded Snacks/Cereals

Extrusion cooked snacks, pasta and cereals often include emulsifiers in their formulas. Gelatinization of the starch occurs during the cooking/extrusion step. Monoglycerides and SSL have been found to reduce the energy required for the extrusion and to produce a desirable texture in the final product. Monoglycerides are added to improve the appearance and smoothness of the extrudate and produce a finer pore structure. Use levels are 0.25–0.5% of the starch weight and is added at the dough make up stage.

10.8.4 Fat-Free Bakery Products

Fat-free and low-fat foods are marketed in almost every segment of the food industry. In most instances, there is no single solution for removal of fats from the formulation. Skillful formulation using fat replacers, emulsifiers, bulking agents, flavors and other ingredients have been applied to fat replacement.

Low-fat and fat-free cakes have been produced using additional emulsifiers in conjunction with starch-based replacers and gums or hydrocolloids for moisture retention and functionality. PGME and DATEM have proved to be particularly useful.

Emulsifiers are not generally regarded as fat substitutes or replacers. Emulsifiers affect the texture and mouthfeel by their surface activity. The caloric value of emulsifiers varies depending on their composition and digestibility. They tend to have fat-like properties through their hydration and dispersing effects in processed foods. The general function of emulsifiers in low-fat and no-fat applications are:

- Prevent separation of components.
- Reduce size of fat globules and improve dispersion of remaining fat.
- Provide fat sparing action.
- Provide texture perception of higher fat contents.
- Texturize and provide lubricity.
- Complex with starches and proteins.

Mono- and diglycerides are the most used emulsifiers. Distilled monoglycerides have lower calories compared to the lower mono content preparations. Other emulsifiers in reduced fat products include the polysorbates, DATEM, polyglycerol esters and sorbitan esters. Emulsifiers used in products having sucrose esters and mixed esters of short and long chain tri-ester replacers are very likely similar to that utilizing traditional caloric versions.

10.8.5 Gluten-Free Bakery Products

Celiac disease is an auto-immune disorder caused by an intolerance to gluten, a structural protein in wheat. It affects approximately 1% of the population in the Western World (Catassi et al. 2010). An additional sub-population regard a gluten-free diet as healthy. Gluten-free baked goods have seen a dramatic rise in interest over the past decade or so as consumers seek gluten-free items as a lifestyle choice (Masure et al. 2016). Early commercial products tended to crumble, dry out, and exhibit off-flavors. In the recent review by Roman et al. (2019), in a survey of emulsifiers used in gluten-free breads currently on the market, 28% used mono- and diglycerides (MDG), 9% used lecithin, 4% used SSL, 3% used DATEM, and 6% used other emulsifiers. Here, the purpose of the emulsifier is not to strengthen the gluten network, but primarily to prevent staling. Thus, DATEM is not so commonly used since its primary function is dough strengthening and MDG is preferred instead. Other functions of the emulsifier might include reducing surface tension to help stabilize air cells and improving grain structure.

Ingredient statements on gluten-free bakery products are very complex and include other surface-active ingredients. Egg and milk proteins have a significant number of lipophilic amino acid residues that migrate to oil droplets and air cells. Milk proteins are not common because they can contain lactose, which is also not tolerated well by some. Further, some hydrocolloids contain proteins that are

surface-active. All of these molecules can interact with divalent metals, acids, electrolytes, and one another in a complex dough/bread matrix. Sorting out these interactions will be a challenge for many future years. Further work is needed to clarify the role of different emulsifiers on attributes of gluten-free baked goods.

10.8.6 Other Applications

Emulsifiers also find various other applications in the baking world. These range from icings, to release agents, to emulsifier-based shortenings.

10.8.6.1 Cream Icings

Cream icings are prepared by creaming sugar with fat, then adding flavor, egg white and perhaps a small amount of water. The emulsified shortenings used contain 2–3% alpha-monoglyceride. PS-60 at 0.5% is included in some icings to assist in aeration. PGME, when incorporated into the shortening, produces icings with excellent gloss and gloss retention.

10.8.6.2 Release Agents

A separate application of emulsifiers in bakery products, although not incorporated in the dough, is release agents or pan sprays. Lecithin is the primary emulsifier used. Often the pan sprays are formulated with an oil in combination with mold inhibitors and lecithin. Lecithin levels of 1–6% are added. Modified lecithins that possess improved heat stability may be used. The pan spray may simply be brushed on or sprayed to achieve a thin film promoting easy release of baked products from pans or belts.

10.8.6.3 Trans-Free Shortening

Consumption of trans fatty acids have negative health consequences. As much as 40% of the trans fatty acids in the diet are from shortenings used in bakery product. These originate from the partial hydrogenation process used to produce the shortening. Partial hydrogenation results in oxidatively stable products with the desired properties of shortenings. Alternatives to partially hydrogenated shortenings include simple blending of commodity oils with fully hydrogenated hard fats, interesterified products, use of naturally saturated oils such as palm oil and fractions and trait modified oils (Cowan and Husum 2004). For those applications using trans-free shortening, the traditional emulsifiers such as GMS function similarly. Shelf stability of the finished products, particularly with the trait modified oils, seems to not be affected.

10.8.6.4 Oleogels

A recent trend in many fat-based foods is to replace saturated fats in semi-solid fat-based products (e.g., shortening) with oleogels, solid-like materials based on solidification of a liquid oil with some organic gelator. Gelators can take many forms, including low-molecular weight organogelators (LMOG) and polymeric gelators (Co and Marangoni 2018). The LMOG include emulsifiers such as fatty acids, monoglycerides, sorbitan esters, and lecithin. Oleogels are now available that quite closely mimic the pertinent properties of shortenings and other fats. They have been tested in a variety of foods, including baked goods, primarily in an effort to reduce saturated or total fat levels (Patel and Dewettinck 2016).

For example, Blake and Marangoni (2015) evaluated structured-lipid oleogels based on monoglycerides of different chain length with different vegetable oils as replacement for shortening in puff pastry. They concluded that "the findings were very promising", suggesting that a structured-lipid olegel based on monoglycerides could be used to replace regular shortening in puff pastries. Along a similar line, Meng et al. (2019) evaluated oleogels based on SSL for use in bread as a replacement for traditional fats. They replaced margarine in bread with an oleogel made with 9% SSL in sunflower oil to reduce saturated fat content by 30%. They argued that the structure of the oleogel matched that of triglycerides in margarine well enough to make bread with acceptable loaf volume and other physical and sensory characteristics.

As seen here, there is significant potential for emulsifier-based oleogels to serve as fat replacement in many baked goods. Further work is warranted to evaluate broader applications.

10.9 Summary

The market for emulsifiers for bakery products continues to increase. As with many industries, bakeries have undergone consolidation. Fewer producers have placed greater requirements on the final products such as longer distribution, longer time from production to consumption, greater stability and shelf-life. The function of the emulsifier is of ever greater importance. Growth in food service increases the need for bakery products having desirable sensory and performance characteristics to meet the demands of tomorrow's market place. New trends and consumer preferences are likely to make the role of emulsifiers in baked goods even more important in the future.

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Chapter 11
Emulsifiers in Confectionery

Richard W. Hartel and Hassan Firoozmand

11.1 Introduction

Confectioneries are multi-phase systems, rich in carbohydrates and sugars, containing various amounts of fat/oil and relatively small proportion of proteins, either from animal or plant sources. In such an inhomogeneous food system, different components constitute diverse interfaces with different hydrophilic-hydrophobic affinities. Therefore, emulsifiers can play a critical role in confectionery products, either in processing stage, or storage period, or even during consumption. In processing, emulsifiers help to break down the fat phase into small fat globules in products such as caramel and toffee. In fat-continuous confections, namely chocolate and coating, emulsifiers provide viscosity control, ease in mixing and coating processes, and also may impart advantages in storage by influencing fat crystallization, inhibiting bloom, and moderating polymorphic transformation. Also, during consumer consumption, they improve the sensory experience; for instance, in toffee, emulsifiers provide lubrication effect in the oral cavity, ease of chewing and swallowing. Similarly, in chewing and bubble gum, emulsifiers act to plasticize gum base (advantage while processing) and provide hydration effect during chewing.

Insoluble phases in confections, such as oil and water or most protein and polysaccharide with concentration higher than 4–5 wt%, have a natural tendency to minimize their contact by repelling each other and separating out into distinct phases. Components separating out in most food systems are undesirable at any point, either in processing (for instance, in a toffee mix during cooking, oiling under agitation due to uncontrolled fat droplet coalescence), or after processing during distribution and

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storage, because phase break-up degrades the physical qualities of the product such as appearance, taste and texture. Phase dissociation must be controlled to obtain a stable quasi-homogeneous state for certain period of time before consumption (best before date). This can be achieved by the combination of appropriate processing technique and proper emulsifier usage. However, the nature and the amount of the emulsifiers by itself often influence the physical qualities of the food product.

Emulsifiers behave as surfactants in most confections, their presence mostly affect the continuous phase and they deliver different functionality in different confectionery products. For instance, lecithin impacts fat crystallization kinetics in chocolate, it acts as crystallization retarder, but in toffee it acts as a surfactant, breaking down the oil phase into small droplets. Therefore, emulsifiers provide a specific benefit in each product. That is, many diverse emulsifiers from different classes described in this book have found their way into confectionery products. These include lecithin and modified lecithins (YN) as well as phosphated monoglycerides, glycerol monostearate, polyglycerol esters including polyglycerol polyricinoleate (PGPR), sorbitan esters, polysorbates, lactic acid and tartaric acid derivatives of monoglycerides, acetylated monoglycerides, sucrose esters and propylene glycol monoesters. The common features that make the aforementioned surfactants as functional emulsifying agents are their amphiphilic nature, they possess both lipophilic and hydrophilic properties. This characteristic is quantified by the balance between hydrophilic and lipophilic moieties in their molecules, in the so-called HLB numbers. HLB indicates a predominant property of a given emulsifier and is identified by a number from 0 to 20. An emulsifier with a lower HLB number typically has a small hydrophilic group and has higher tendency to reside at oil phase and therefore, it is more lipophilic. On the other side, an emulsifier with higher HLB number with larger hydrophilic group has greater affinity to the aqueous phase. For instance, lecithin has an HLB number of 4 and therefore, it resides in the oil phase in a confectionery product such as chocolate. In contrast, polysorbate 60, with an HLB number of 15, is quite soluble in water, and consequently it resides in the syrup phase of toffees and caramels. To make the most of the emulsifiers in a food or confectionery product, usually a combination of both low and high HLB numbers is added to the product; thus, a more stable product is obtained.

In this chapter, the more common categories of confectionery that use emulsifiers are described, along with technical reviews of the available knowledge related to the most optimal emulsifier types and their applications and benefits.

11.2 Emulsifiers in Chocolate and Compound Coatings

The use of emulsifiers in chocolate and compound coatings is perhaps one the best documented in the literature of any of the applications in confectionery. In chocolate, lecithin and PGPR are the primary emulsifiers, but in compound coatings numerous other emulsifiers are used. For the most part, emulsifiers provide control over flow properties when used in chocolates and coatings, although they may have other effects as well.

It is well known that the addition of low levels (tenths of a percent) of emulsifiers can reduce viscosity equivalent to several percent addition of more fat (e.g., cocoa butter). That is, emulsifiers are cost-saving ingredients in chocolate. Nonetheless, emulsifiers may have other effects as well such as controlling fat crystallization and polymorphism. However, the different emulsifiers influence flow properties in different ways and it is important to understand the mechanisms of these effects in order to optimize their use.

Melted chocolate and compound coatings are dispersions of solid particles including sugar granules, milk solids, and cocoa solids, in a continuous liquid fat phase. Both chocolate and compound coatings contain about 30–35% fat, with the rest being mostly particles. In chocolate, the fat is cocoa butter, which comes directly from the crushing of cocoa nibs, whereas in compound coatings, the fat comes from vegetable oils added to the formula. Chocolates and coatings also contain a small amount of moisture (about 0.5%), introduced indirectly via the sugar or other solid ingredients. The presence of these solid particles and moisture in chocolate and compound coatings causes their flow behavior and viscosity to deviate from true Newtonian flow. When the solid particles in chocolate and compound coatings flow past each other in a shear field, there is a hydrophilic attraction of their surfaces towards each other. The resultant internal friction causes the apparent viscosity of the material to vary according to the applied shear rate (non-Newtonian behavior). The flow properties of chocolate and compound coatings are critical to processing and applications, because they always have to flow to either fill a mold without defects or trapping air bubbles or cover a candy piece with a thin, even coat. The flow behavior of the coating is dependent on both the nature of the continuous liquid phase (the fat and fat-soluble ingredients) and the nature of the dispersed particulate phase. The dispersed phase volume (mass of particulates), their size and size distribution, and their shape and surface characteristics all impact the rheological behavior of chocolate and coatings.

Molten chocolate and coatings are non-Newtonian fluids, exhibiting shear-thinning behavior. That is, the apparent viscosity of chocolate decreases as the shear rate increases. Chocolate appears thinner when stirred or pumped at higher rates. Conventionally, the rheological properties of chocolate are expressed by the Casson model (Seguine 1988).

$$\left(\sigma\right)^{1/2} = \left(\sigma_0\right)^{1/2} + \left(\eta_c\right)^{1/2} \left(\dot{\gamma}\right)^{1/2} \tag{11.1}$$

Here,

σ is shear stress,
σ_0 is Casson yield value,
η_c is Casson plastic viscosity,
$\dot{\gamma}$ is shear rate.

The rheological properties of chocolates are defined by the Casson parameters, plastic viscosity, η_c and yield value, σ_0. "Yield value" is the force required to start the mass of liquid chocolate to move, whereas "plastic viscosity" defines the force required to keep liquid chocolate flowing once it has started moving. Plastic viscosity and yield value are often combined in a single value called "apparent viscosity",

technically defined as shear stress over shear rate. However, this simplification results in a loss of detail since chocolates with equal apparent viscosities can have different yield values and different plastic viscosities. Furthermore, independent control over yield value and plastic viscosity are often needed to design chocolates and coatings for specific tasks.

Coatings can always be made more fluid for better control by adding more cocoa butter or vegetable fat to the mix, but as these are the more costly ingredients in coatings, this is often an unattractive solution. Better by far is to add a surfactant like lecithin or PGPR to reduce coating viscosity. Both plastic viscosity and yield value can be decreased by the use of specific surfactants and this enables the chocolate manufacturer to have greater control of cocoa butter or levels.

11.2.1 Lecithin

Lecithin commonly refers to a multi-compound mixture of naturally occurring phospholipids (Joshi et al. 2006). As nature's principal surface-active agents, lecithin is found in living organisms and is essential to life (Szuhaj and van Nieuwenhuyzen 2003). Lecithin is commercially produced mainly from soybean, but also from sunflower seeds, by solvent extraction and precipitation. Soy lecithin is a light brown fluid that contains approximately 65% acetone insoluble phosphatides and 35% soybean oil. An average chemical composition of soy lecithin is given in Table 11.1. However Geisler (1991) lists nearly 30 different components of soy lecithin and generally differentiates the components based on their acetone solubility. The acetone soluble portion contains primarily soybean oil, fatty acids, glucosides and sterols. The acetone-insoluble fraction contains the phospholipids as well as any carbohydrates bound to the phospholipids.

The surface-active components of lecithin are amphiphilic molecules that exhibit both lipophilic and hydrophilic properties. The chemical structure of one of the main components of lecithin (phosphatidyl choline) is shown schematically in Fig. 11.1. The phosphatidyl group, the hydrophilic component of the lecithin molecule, prefers to be in the aqueous phase, whereas the two fatty acid chains are lipophilic and orient into a lipid phase of a food. Depending on the source, the fatty acid chains may be either saturated (palmitic or stearic) or unsaturated (oleic or linoleic). In chocolate and coatings, the hydrophilic part of the lecithin molecule

Table 11.1 Average composition (%) of soy lecithin

Soybean oil	35
Phosphatidyl choline	18
Phosphatidyl ethanolamine	15
Phosphatidyl inositol	11
Other phosphatides and polar lipids	9
Carbohydrates, e.g., sterols	12

From Minifie (1980)

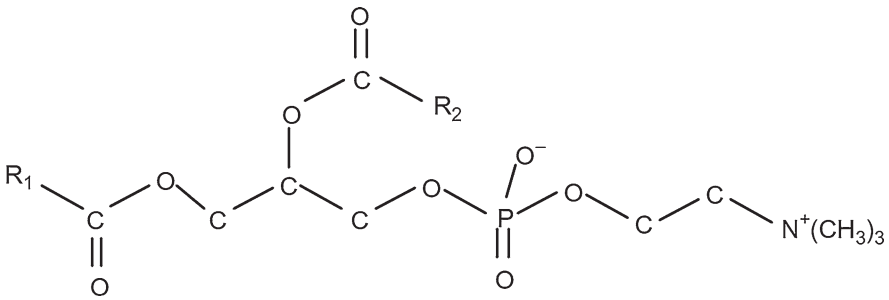

Fig. 11.1 Molecular structure of phosphatidylcholine. R_1 and R_2 are the alkyl chains

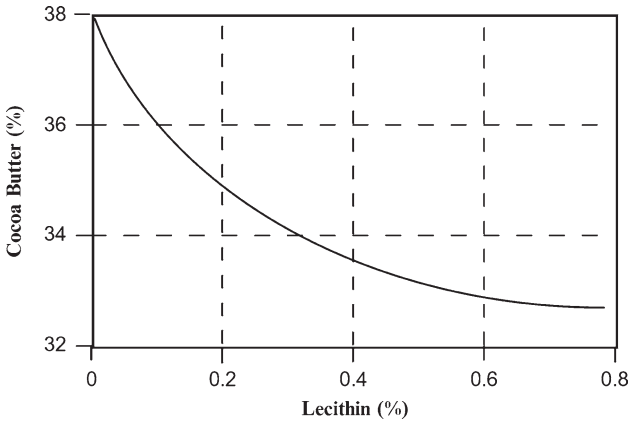

Fig. 11.2 Effect of addition of lecithin on fat content required to maintain constant viscosity in dark enrobing chocolate. (After Minifie 1980)

orients at the hydrophilic sugar crystal surface, with the fatty acid chains oriented into the continuous fat phase. Recently, Kindlein et al. (2018) conducted molecular dynamics simulations on lecithin phosphatides at a sucrose crystal surface in a lipid melt. Results suggested that the different phosphatides in lecithin form a monolayer at the sugar crystal surface with the same concentration as found in the lecithin—no separation of phosphatides was observed.

Due to its surface-active nature, particularly at the hydrophilic sugar crystal surface, lecithin provides a significant reduction in viscosity of chocolate and coatings. For example, addition of 0.5% lecithin to a coating gives the same viscosity reduction effect as addition of 5% cocoa butter or vegetable fat (Minifie 1980). Lecithin allows coating users to operate efficiently at much lower fat contents than would otherwise be the case (Fig. 11.2).

Lecithin addition up to about 0.6–0.8% results in a decrease in both Casson yield value and plastic viscosity (and thus, a decrease in apparent viscosity). However, higher addition levels actually cause apparent viscosity to increase again. This thickening effect is thought to be due to weak interaction forces such as

hydrogen bonding between lecithin molecules and its surrounding (Pedersen 2014). Higher addition levels result in an increase in Casson yield stress with no further reduction in Casson plastic viscosity; thus, the apparent viscosity increases (Chevalley 1988).

It has been shown that Casson yield stress begins to increase in chocolate with 33.5% fat content (1.1% water) when lecithin addition level is about 0.5% (Chevalley 1988) and about 0.6% lecithin for dark chocolate containing 36% fat sweetened with sucrose (Nebesny and Zyzelewicz 2005). In contrast, the increase in Casson yield value began at about 0.4% lecithin addition in a chocolate with 39.5% fat (and 0.8% water) (Chevalley 1988) as well as for milk chocolate containing 34% fat sweetened with isomalt (Nebesny and Zyzelewicz 2005). Presumably, these differences are more due to water content rather than to fat content or type. Lecithin used in excessive amounts may also produce certain negative effects, such as softening of chocolate and increase of crystallization time (Jeffery 1991). This is because the chemical structure of lecithin is very different from cocoa butter or vegetable fats and it can interfere with the crystallization process in the fat phase. Also, in higher amount, lecithin can negatively affect the sensory attributes of chocolates, because lecithin imparts a characteristic and unpleasant taste and aroma (Nebesny and Zyzelewicz 2005).

A potential explanation of the mechanism by which lecithin reduces intra-particle friction was offered by Harris (1968). Moisture present in chocolate and compound coatings adheres to the surface of sugar particles to give them a syrupy, tacky surface that in turn increases friction between the sugar grains. When lecithin is introduced, the hydrophilic functional group in lecithin attaches itself to the sugar surface while the lipophilic group is left to project out into the surrounding oil phase. This enables the particles to slip more easily over each other reducing the viscosity. Rousset et al. (2002) studied the action of lecithin at the sugar crystal interface by inverse gas chromatography. Their results show that adsorption of lecithin at the sugar crystal surface increases the lipophilic character of that surface, which decreases sucrose-sucrose interactions. This effect is demonstrated in Fig. 11.3 where the viscosity reducing effect of lecithin is only seen where sugar is present in the formula. Furthermore, it is shown that in chocolate mass containing fiber as sucrose replacer, addition of lecithin did not result in a decrease in the Casson yield value (Bonarius et al. 2014).

The increase in viscosity (yield value) at higher lecithin levels has not been adequately explained. Geisler (1991) suggests that this effect is due to lecithin multilayer formation at the interface. Also, lecithin micelle formation in the continuous phase is linked to increase in yield value (Afoakwa et al. 2007; Vernier 1997). The recent modeling study of Kindlein et al. (2018) suggested that higher concentrations of lecithin causes formation of "wormlike reversed micelles or highly structured lyotropic liquid crystalline phases." These presumably create additional structures that can form networks while at rest to increase the force required to initiate flow.

The viscosity of a dispersion of particles in liquid oil is actually a function of numerous parameters, above and beyond the emulsifier used. The nature of the solid dispersion affects viscosity (Chevalley 1988), including parameters such as dispersed

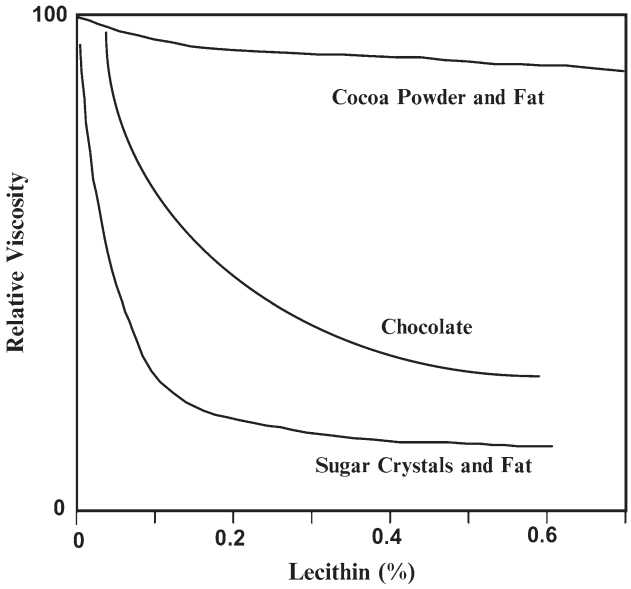

Fig. 11.3 Effects of cocoa particles and sugar crystals on viscosity of suspensions, as compared to the behavior of chocolate. (After Minifie 1980)

phase volume, particle size and shape, and surface characteristics. Also, the type of oil used and its level of minor impurities, especially those that are surface-active, can affect flow properties. Arnold et al. (2013), by using the force/distance function of atomic force microscopy (AFM), reported that lecithin reduced the adhesion forces between dispersed sugar particles in soybean oil. This effect was more pronounced when sugar particles were suspended in medium chain triglycerides.

Babin et al. (2005) studied model systems of sugar crystals dispersed in different fats. For the same level of dispersed phase solid particles, viscosity was different in different oils (no emulsifier added). Viscosity (and the Casson parameters) in cocoa butter was always the lowest of the fats studied, whereas the highest viscosity was found in palm kernel oil. Soybean oil and milk fat had intermediate viscosity. Addition of lecithin always reduced viscosity, but the effects were different in different oils. The greatest effect in decreasing viscosity was found in palm kernel oil. The viscosity results were found to correlate well with differences in sedimentation volumes of sugar particles in each oil. Samples that had highest viscosity also had highest sedimentation volume, indicating that particle attractive forces were strong. Differences in sedimentation volume (and hence viscosity) were also seen when the oils were purified by contact with either activated charcoal or Florisil. In general, lower sediment volume (more compact sediment with fewer aggregated particles) was found after the oils were purified, although some differences were observed among the fats. Sedimentation volumes decreased for both activated charcoal and Florisil treatment of soybean oil, whereas the sedimentation volume for cocoa butter did not change at all after either treatment. For palm kernel oil, sedimentation

volume went down after Florisil treatment but did not change after treatment with activated charcoal. Interestingly, the sedimentation volume for milk fat went down after activated charcoal treatment, but increased after treatment with Florisil. Treatment of the oils with either activated charcoal or Florisil will remove certain types of minor impurities, including water and polar lipids. However, no compositional data was provided from which to understand the molecular basis for changes in sedimentation volume (and therefore, of viscosity).

Although soy lecithin is the main source used in chocolates, recent interest in alternatives has been increasing. Lecithins are now available from sunflower and canola, and others have evaluated phospholipids extracted from *Camelina sativa* seeds (Belayneh et al. 2018) or dairy sources (Zhu and Damodaran 2013; Price et al. 2018). Depending on the source and extraction method, the specific phosphatide content and distribution may differ. For example, dairy lecithin contains significant levels of sphingomyelin, not found in plant sources. Böhme et al. (2016) compared properties of chocolates made with soy, sunflower and canola lecithins. Despite the differences in phosphatide content and distribution, no differences in rheological or sensory aspects were observed among the chocolates.

In addition to its effect on viscosity, lecithin also affects fat crystallization kinetics in chocolate (Savage and Dimick 1995). In one study, addition of 0.5% lecithin reduced the induction time for crystallization and increased the rate of crystal content increase in a model system of cocoa butter and sugar solids (Svanberg et al. 2011). However, in another study, addition of 0.2% lecithin produced a slight increase in induction time in a similar model system (Dhonsi and Stapley 2006). Further work would be needed to verify the exact conditions under which lecithin influences cocoa butter crystallization.

Lecithin is usually added late in the chocolate or compound-making process since it can be absorbed by cocoa particles during grinding and mixing, thereby losing its effectiveness. In some cases, a small amount of lecithin is added to the mixed ingredients prior to roller refining to aid in the grinding process, but the remaining portion is added just before the end of the conching process. This provides the maximum liquification of the chocolate or compound coating at minimum fat content. Lecithin also has the benefit of protecting coatings against moisture invasion and sugar granulation, which may occur at temperatures above 60 °C when stored in bulk form.

11.2.2 Synthetic Lecithin

Synthetic lecithins are made by reacting mono, di and triglycerides of partially hydrogenated rapeseed oil or other liquid vegetable oil with phosphorous pentoxide to produce phosphatidic acids. Neutralization with ammonia or caustic soda results in an ammonium or sodium salt. These surfactants are often given the name synthetic lecithins or sometimes YN lecithin. They have a neutral flavor, have a slightly greater effect of reducing chocolate viscosity than lecithin extracted from soya, as

seen in Fig. 11.4, and can be used at higher dosage levels than natural lecithin without the negative impact on viscosity (Bonekamp-Nasner 1992; Kleinert 1976; Nakanishi and Shiomi 1971). They are not generally used in the United States because of cost (Geisler 1991).

YN lecithin is also claimed to reduce the thickening of chocolate and compound coatings due to moisture and overheating (Bradford 1976). A comparison between lecithin at 0.3%, YN at 0.3%, and cocoa butter added at 5% to chocolate gave similar overall viscosity readings (see Fig. 11.5), but a calculation of the Casson yield values showed that YN produced significantly lower values than with the other systems. While addition of lecithin at more than 0.4% increases the yield value, higher levels of emulsifier YN continues to reduce plastic viscosity with constant yield value (Pedersen 2014). The viscosity-reducing effect of YN is reportedly less in milk-free coatings than with milk coatings (Hogenbirk 1989; Kleinert 1976). Milk coatings generally have higher viscosities than milk-free coatings due to the effect of milk solids/fat/emulsifier interactions. These interactions result in higher viscosities compared to coatings containing only cocoa solids and sugar for surfactant adsorption. Details of how these interactions occur are absent from the literature.

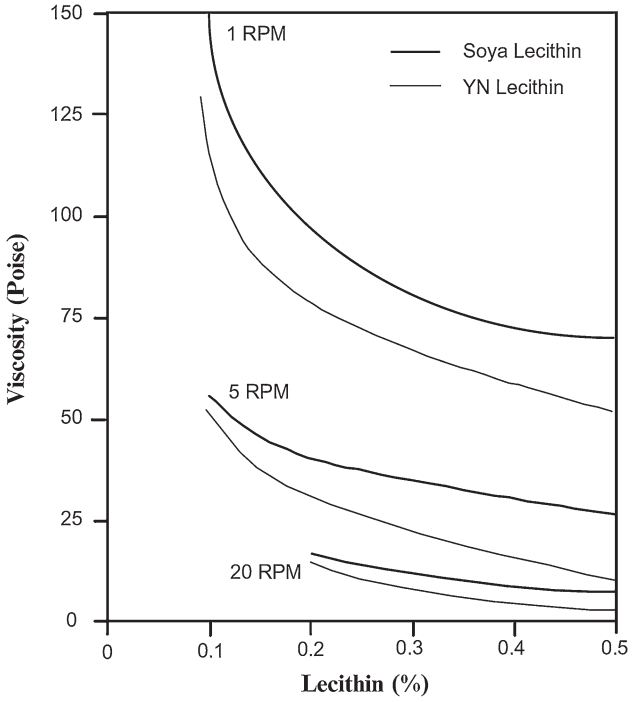

Fig. 11.4 Brookfield viscosity measurements of milk chocolate at three levels of shear (RPM). Comparison of effect of soya lecithin and YN lecithin. (After Minifie 1980)

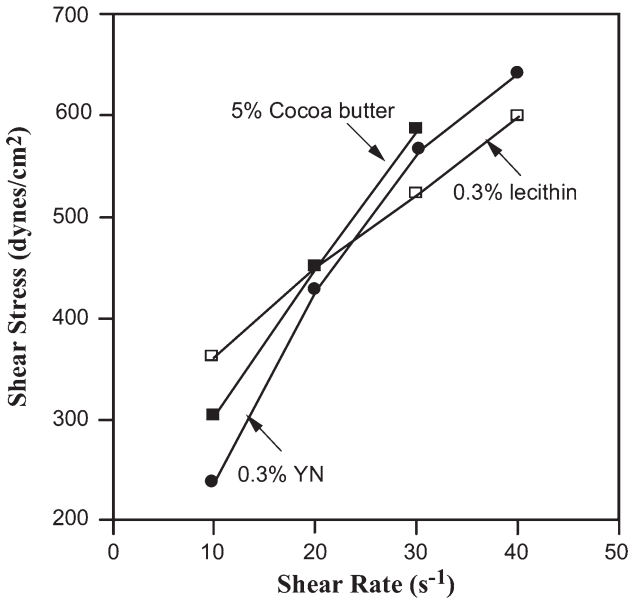

Fig. 11.5 Viscosity plot comparing lecithin, YN and cocoa butter. (Adapted from Bradford 1976)

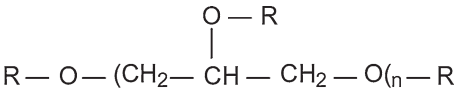

Fig. 11.6 Chemical structure of polyglycerol polyricinoleate (PGPR), where R = H or a fatty acyl group derived from poly condensed ricinoleic acid and n = the degree of polymerization of glycerol

11.2.3 Polyglycerol Polyricinoleate PGPR

PGPR is a surfactant used in the chocolate and compound industries in Europe and other parts of the world, and has recently been approved for use in the United States. It has a unique role to play in modifying the viscosity behavior of chocolate coatings. It is made by reacting polyglycerol with castor oil fatty acids under vacuum. The resultant material is a colorless, free-flowing fluid with little or no odor. PGPR is also claimed to be a moisture scavenger in chocolate and compound coatings (Garti and Yano 2001) preventing thickening of coatings over time (Application Notes Admul WOL, Quest International). Its chemical structure, in general form, is shown in Fig. 11.6.

A number of studies have been published that compare the effects of PGPR with lecithin and YN. Most conclude that PGPR, when added to chocolate or compound coatings at 0.5% or less, can reduce the coating yield value to almost zero (Application Notes Admul WOL, Quest International; Bradford 1976). The practical benefit of such a feature is that in a chocolate bar molding operation, PGPR

addition would allow the chocolate to flow easily into even complicated mold shapes without entrapping air bubbles and also flow around inclusions. Furthermore, the opportunity exists to reduce the fat content of the chocolate as well as the cost of chocolate formulations. It is possible to reduce the total fat content by 3% from 34% to 31% and achieve the same flow properties by addition of 0.15% to 0.25% PGPR to the chocolate mass (Poulsen 2013).

A typical comparison of lecithin and PGPR additions to a milk chocolate with 35.5% fat content is shown in Table 11.2, and a similar comparison in dark chocolate is shown in Table 11.3 (Application Notes Admul WOL, Quest International). In milk chocolate, it is possible to reduce the yield value to almost zero through addition of PGPR. Rector (2000) observed a similar decrease in Casson yield value when using PGPR in chocolates. The combination of lecithin and PGPR also allows the plastic viscosity to be decreased (Table 11.2). In dark or semi-sweet chocolate, the effect of PGPR on plastic viscosity is slight while it can reduce yield values to very low values at 0.5% addition (Table 11.3) Schantz and Rohm (2005) suggest that the most efficient mixtures of lecithin and PGPR for reducing yield stress in both milk and dark chocolates was 30% lecithin and 70% PGPR. Lowest plastic viscosity values were found for 50:50 mixtures of lecithin and PGPR in dark chocolate, whereas the ratio 75:25 lecithin to PGPR gave the lowest plastic viscosity for milk chocolate. They concluded by stating that yield stress and plastic viscosity could be tailored to suit a specific application by proper choice of the lecithin to PGPR ratio.

Table 11.2 Casson plastic viscosities and yield values of a milk chocolate when cocoa butter, lecithin and PGPR are added

Addition	Amount	Casson plastic viscosity (poise)	Casson yield value (dyn/cm^2)
Cocoa Butter	0.0	45	110
	1.0	29.8	97
	2.0	26.5	62
	4.0	16.3	58
	5.0	15.3	58
Lecithin	0.05	30.0	79
	0.1	26.7	54
	0.2	20.0	40
	0.4	15.6	37
PGPR	0.075	30.0	86
	0.175	29.2	38.5
	0.3	26.8	22
	0.5	30.5	2.5
	0.6	32.0	2.0
Lecithin + PGPR	0.1	14.1	34
	0.2	13.4	32
	0.3	12.7	29

Table 11.3 Casson plastic viscosities and yield values of a dark chocolate when cocoa butter, lecithin and PGPR are added

Addition	Amount	Casson plastic viscosity (poise)	Casson yield value (dyne/cm^2)
Lecithin	0.3	18.5	155
	0.7	17.1	221
	0.97	14.4	297
	1.3	12.4	282
PGPR	0.0	12.9	199
	0.1	12.5	151
	0.2	14.8	82
	0.5	14.9	13
	1.0	15.9	0

PGPR is also claimed to be advantageous for use in ice cream coatings since it allows low apparent viscosities in the presence of low levels of moisture (Bamford et al. 1970). Also claimed is PGPR's beneficial effect on fat phase crystallization leading to easier tempering, improved texture and longer shelf life of coatings (Application Notes Admul WOL, Quest International). However it has been reported that in comparison with lecithin and emulsifier YN, addition of PGPR accelerated the crystallization process and could therefore be susceptible to over-crystallization (Schantz and Linke 2001). The viscosity-reducing properties of PGPR lead to significantly reduced viscosity at temper and a level of temper, as measured by a temper meter, which is easier to maintain over long periods in an enrober without significant recirculation of chocolate via melt-out and retempering circuits (personal communication).

PGPR's most recognized benefit remains that of fat reduction. Chocolate manufacturers claim that a blend of 0.5% lecithin and 0.2% PGPR allows cocoa butter reductions of approximately 8%. Also the blend of PGPR plus lecithin is very effective to stabilize water-in-cocoa butter emulsions (Rivas et al. 2016).

11.3 Anti-bloom Agents in Chocolate and Compound Coatings

Fat bloom in chocolate and compound coatings is due to the appearance of fat crystals emanating from the surface (Lonchampt and Hartel 2004; Timms 2003). A bloomed chocolate or coating is characterized by an initial loss of surface gloss, followed by appearance of a white or gray haze at the surface. Fat bloom can occur for many reasons, and may be related to improper processing conditions, composition and storage conditions. Numerous references can be found documenting the effects of various emulsifiers on fat bloom in chocolates and compound coatings, although our understanding of the complex phenomena that lead to bloom formation is still incomplete (Lonchampt and Hartel 2004).

One of the main goals during processing of chocolate is to ensure that the cocoa butter crystallizes in the correct crystal form or polymorph (Timms 2003). Cocoa butter has several different polymorphic forms that have melting points ranging from 17 to 35 °C. The forms are represented by the Greek letters γ, α, β′ and β, listed in increasing order of stability. As the polymorphic form increases in stability, it also increases in melting point. To make chocolate in the familiar glossy, fast-melting form with good snap, it is necessary to crystallize the cocoa butter in a high-melting, reasonably stable polymorph, sometimes called the β V form. This form of cocoa butter is also needed to ensure good contraction in molded products and the long bloom-free shelf life expected for good quality chocolate goods. However, the β V form is not the most stable polymorph for cocoa butter, and it slowly converts to the most stable β VI form.

Fat bloom can be caused by a number of different mechanisms.

1. If chocolate is not preconditioned (tempered) correctly such that insufficient concentration of seeds in the β form is present in the crystallizing chocolate mass, this leads to a higher level of less stable β′ forms in the chocolate mass, which later transform to the more stable β form. In fact, studies show that this transformation from β′ form to β form is the result of, rather than the cause of, bloom formation (Wang et al. 2010). This transformation also causes the chocolate coating or bar to contract and squeeze liquid fat to the surface. Chocolate contains liquid fat even at room temperature, where cocoa butter attains a maximum solid fat content of approximately 75–85%. This liquid fat at the surface crystallizes in an uncontrolled fashion and is a mixture of β, β′ and even possibly some α forms.
2. When chocolate is tempered correctly, but subjected to wide temperature variations in storage and distribution, partial melting and re-solidification of the chocolate occurs, leading to bloom formation. Under these conditions, uncontrolled recrystallization takes place and extensive bloom can occur. This kind of change is often referred to as heat damage and the product is classified as not heat resistant.
3. In molded bars that contain peanuts or other nutmeats as solid inclusions, or in enrobed products that have centers containing quantities of soft vegetable oil or dairy butter oil, this oil can "migrate" from the center to the chocolate shell. The soft oil will cause the chocolate to become soft as the cocoa butter dissolves in the oil. This will cause severe damage to the product due to physical handling prior to consumption or due to discoloration and bloom of the chocolate shell, which will now be far more heat sensitive. Oil migration triggers the recrystallization of cocoa butter to the β VI polymorph, which protrude through the chocolate surface and cause a grayer and duller appearance (Clercq et al. 2014).
4. Long-term changes in cocoa butter crystal structure via V–VI transitions can also be a cause of bloom in some cases.

In all the cases above, the negative impact of uncontrolled crystallization is discoloration and fat bloom. This phenomenon is also seen in compound coatings based on other vegetable fats, although there is some question whether the same mechanisms apply (Lonchampt and Hartel 2004). Since many compound coating fats (e.g., palm

kernel oil) have long-term stability in the β′ polymorph, yet still undergo bloom formation during storage, it has been postulated that different mechanisms are responsible for bloom formation in coatings. However, palm kernel oil actually transforms to a β form over long times (Timms 2003), and the presence of a polymorph has been associated with bloom in compound coatings (Talbot et al. 2005). Other lipids, such as minor lipids from milk fat, even at the low concentrations typically found in nature, affected the crystallization of milk fat–CB blends, impacted the chocolate microstructure, and affected bloom development in chocolate. Both removing the minor lipids from milk fat and doubling the level of minor lipids from milk fat resulted in longer nucleation onset time, slower crystallization rate, and rapid bloom development in chocolate. Removal of minor lipids resulted in the formation of irregular primary and secondary crystals with inclusions of liquid fat, whereas the crystals were spherical and uniform in shape in the presence of minor lipids (Tietz and Hartel 2000). Milk fat at 5% can reduce the crystallization (bloom formation) in chocolate, at 7.5% (milk fat) the degree of inhibition is even greater, but unacceptably the chocolate becomes softer (Sonwai and Rousseau 2010). Emulsifiers also help control the rate of crystallization of cocoa butter and other vegetable hard butters, both at time of production and during subsequent storage and distribution. For instance sorbitan monostearate (SMS) at 1.5% increase in the onset of the crystallization temperature (from 19.3 to 23.8 °C) and doubling the yield value (consistency) of pure cocoa butter (Masuchi et al. 2014).

11.3.1 Sorbitan Tristearate (STS)

STS is an emulsifier often associated with bloom prevention; it is claimed that when added to chocolate in the liquid state at 2% or even at 0.5 wt% concentration (Rosales et al. 2017) it slows down the crystallization rate of cocoa butter, thereby reducing the concentration of the most unstable α form. The more stable β′ form is still produced, but this transforms into the β form, thus deterring bloom (Anon 1991b). In this way, STS behaves as a crystal modifier.

However, STS has also been shown to have an effect on the polymorphic transition from β V to β VI. Garti et al. (1986) showed that STS is particularly effective at blocking this V to VI transformation and, hence, preventing bloom even after extensive temperature cycling between 20 and 30 °C. Garti et al. (1986) also studied the effects of sorbitan monostearate (SMS) and Polysorbate 60 on cocoa butter polymorphism, but these were only half as effective as STS on preventing bloom. STS is a high melting point emulsifier (about 55 °C) whose structure is more closely related to cocoa butter triglycerides than most other emulsifier types. It is speculated that it is due to this similarity that it cocrystallizes with cocoa butter from the melt and due to its rigid structure, binds the lattice in the β V form. Other more liquid or less triglyceride-like emulsifiers tend to depress the melt point of crystallized cocoa butter, increasing liquidity and promoting form IV to V transformations in preference.

Co-crystallization of STS with cocoa butter is presumably why STS is a more effective anti-bloom agent in solid chocolate than in enrobed chocolate items, where

soft center oils often migrate into the chocolate, dissolve cocoa butter crystals and allow a β′ to β transition. Krog (1977), however, claims that STS locks fats in the less stable β′ form and prevents the transformation to β. Berger (1990) also claims that STS performs well as a bloom inhibitor or gloss enhancer in palm kernel oil based compound coatings used to enrobe cakes by stabilizing the β′ form of the vegetable fat, a situation also observed in several practical cases using lauric coating fats but with much less reliability when using domestic fats such as soybean or cottonseed based coating fats (M. Weyland, personal communication). Such products tend to have longer bloom-free shelf lives in many cases so that the need for anti-bloom additives is not so imperative.

STS is not allowed in chocolate in the United States, but is often found in compound coatings for the benefits it can bring to appearance and stability. STS is more widely accepted as an additive in EC countries.

11.3.2 Sorbitan Monostearate (SMS) and Polysorbate 60

SMS and polysorbate 60 (also known as polyoxyethylene (20) sorbitan monostearate) are also used as anti-bloom agents, especially in compound coatings. They are not as effective as STS but have the advantage of being already accepted by FDA as food grade emulsifiers. They are usually used in combination, where the SMS acts as a crystal modifier and the polysorbate acts as a hydrophilic agent to improve emulsification with saliva and aid flavor release (Dziezak 1988; Lees 1975). SMS, with a melt point of 54 °C, can also be used at high levels in coatings to increase heat resistance; unfortunately, the addition of SMS and the high melting point also cause the coating to become waxy.

Up to 1% of SMS and polysorbate 60 can be added to coatings to improve initial gloss and bloom resistance. The optimum ratio of SMS to polysorbate 60 has been given as 60:40 (Woods 1976). These emulsifiers are claimed to function by forming monomolecular layers of emulsifier on the surface of sugar and cocoa particles, thereby inhibiting the capillary action that causes liquid fat to migrate to the surface and cause bloom. Lecithin is still needed in these systems to control coating viscosity and reduce fat content.

SMS (or Span 60) and polysorbate 60 (or Tween 60) are also generally thought to reduce the rate of fat crystallization, apparently affecting kinetics of crystallization but not the polymorphic behavior (Sonwai et al. 2017); therefore, to develop proper crystal size a suitable tempering system needs to be employed. SMS and polysorbate 60 may be employed in both chocolate and compound coatings with advantage if fast crystallization of the coating would be disadvantageous.

Sometimes, combinations of emulsifiers provide distinct advantages over a single emulsifier, as for the example of PGPR and lecithin noted earlier. For another example, the combination of STS and SMP (sorbitan monopalmitate) in the ratio of 1:1 and at a level of 0.5% was found to be more effective than either STS or SMP alone to prevent oil separation in high oil content (>27.5%) middle eastern confectioneries (Guneser and Zorba 2014).

11.4 Other Emulsifiers Used in Coatings

Mono- and diglycerides are also used as additives to chocolate and compound coatings, often as their purified or distilled forms. They can act as seeding agents especially when in high melting point forms such as glycerol monostearate (GMS). They are more commonly used as anti-bloom agents in lauric-type palm kernel oil compound coatings to extend useful shelf life. A typical usage level would be 0.5%. Berger (1990) claims good results in hydrogenated palm kernel oil coatings when using glyceryl lacto palmitate at 1–5% as a gloss improver; the application was as a coating for a baked product. Moran (1969) found that a polyglycerol ester of stearic acid reduced the viscosity of fat-sugar systems more effectively than lecithin as well as retarded crystallization, improved gloss and gave better demolding.

Lactic acid esters of monoglycerides have also been used to control gloss in compound coatings (Dziezak 1988; Hogenbirk 1989) and to improve demolding performance (Anon 1991a). Lactylated monoglyceride could improve the bloom stability of the chocolate but reduces the sensory attributes of the chocolate; however, the sensory attributes could be achieved by addition of tristearin as crystallization promoter (Jovanovic and Pajin 2004). Woods (1976) describes the use of triglycerol monooleate in compound coatings and chocolate to improve initial gloss and gloss retention, and triglycerol monostearate as a whipping agent to aerate coatings giving them a lighter texture for filling applications. Herzing and Palamidis (1982) describes in detail the types of polyglycerol esters, triglycerol monostearate, octaglycerol monostearate and octaglycerol monooleate, needed to optimize the glossy properties of lauric and nonlauric compound coatings. These emulsifiers are added to the coating fat at up to 6% by weight.

Polyglycerol esters have also been claimed to speed up the setting time of chocolate pan coatings when used at levels of 0.4–0.6% (Player 1986). Hogenbirk (1989) found some degree of viscosity reduction in compound coatings made with mono- and diglycerides, diacetyl tartaric esters of monoglycerides (DATEM), acetylated monoglycerides, and proplylene glycol monoesters. Musser (1980) showed the benefits of adding up to 1.5% DATEM to chocolate and compound coatings to modify viscosity and to improve the rate of fat crystallization. The addition of DATEM to fully lecithinated milk and dark chocolates, and dark sweet coatings, caused a further decrease in viscosity, an effect also observed by Weyland (1994). DATEM also acted as a seeding agent, improved the speed of crystallization and resulted in finer grain and better gloss in molded bars.

11.5 Emulsifiers in Non-chocolate Confectionery

Unlike in chocolate and compound coatings, the continuous phase of sugar confectionery is not lipid, but sugar syrup (in this case, “sugar” means any nutritive carbohydrate sweetener). For this reason, the role of an emulsifier in sugar confectionery is to enable small quantities of lipophilic material to be finely dispersed within a

sugar matrix to achieve a desired effect. This effect may involve the dispersion of fat globules, hydrophobic colors and flavors, or some other fat-soluble ingredient throughout the sugar matrix, or the direct physical interaction of the emulsifier with the sugar phase to achieve the desired textural properties.

A major factor in consumer acceptance of a confection is the "mouthfeel." Vegetable fats and emulsifiers are used to improve texture and lubricate the product to achieve better chewing characteristics. For example, a small amount (a few percent) of fat in a chewy candy provides lubrication both during processing (with high-speed equipment) and consumption (with teeth). A well-chosen surface-active agent can improve this aspect as well as slow down the release of added flavorings. They will affect the viscosity characteristics of the sweet and may even influence the crystal shape present in grained confections. Furthermore, improvement in fat dispersion throughout the confection slows the rate at which the ingredient becomes rancid as the amount presented or migrating to the surface is lessened.

Emulsifiers are commonly found in confectionery products like chewing and bubble gum, caramel, toffee and fudge, starch-based candies like jellies and licorice, and chewy candies.

11.5.1 Chewing Gum

Gum is made of gum base, sweeteners, humectants, and colors, flavors and acids. Gum base, the main functional ingredient of gum, contains numerous components chosen to provide the specific attributes (chewing versus bubble gum, acid or non-acid gum, flavor release, hardness, etc.) desired in gum. Although the composition of gum base is controlled by the Code of Federal Regulations, a wide range of ingredients can be added to provide specific functionality. The primary functional ingredient of gum base is the elastomer, either synthetic or natural, which provides the desired chewy characteristic. However, various modifiers, fillers, plasticizers, softeners, emulsifiers and antioxidants can be added to gum base to provide specific effects and are not required to appear on the product label.

In gum, emulsifiers primarily act to soften the gum base through eutectic interactions with lipid components. Besides softening the gum, emulsifiers provide numerous advantages in gum. They also promote water retention and hydration of the gum base during chewing as well as reducing tack to teeth. Emulsifiers can also act as carriers for colors and flavor, aiding in the dispersion of these important ingredients within the gum base and facilitating their release into the oral cavity during chewing. Further, emulsifiers facilitate bubble formation in bubble gum.

Emulsifiers can be added either during manufacture of gum base or with the other ingredients as gum is formulated. Common emulsifiers added to gum base include monoglycerides, distilled monoglycerides (e.g., glycerol monostearate), and ACETEM. Emulsifiers make up between 2% and 14% of gum base, or 0.5% and 4% on the final weight of gum. The main emulsifier added during gum formulation is lecithin. Up to 1% lecithin can be used to soften chewing gum to the

desired consistency (Patel and Dubina 1989) and can be hydrated or mixed with a vegetable oil or suitable fatty emulsifiers, such as mono- and diglycerides, to aid in dispersion within the chewing gum. Chewing gums prepared in this way have the desirable soft, chewy properties popular in today's top products. Other emulsifiers are also used in chewing gum to provide suitable textural and anti-stick properties to the chewing gum base. These include glyceryl lacto palmitate, sorbitan monostearate, triglycerol monostearate, triglycerol monoshortening, and polysorbates 60, 65 and 80.

Lecithin may also be used to provide a protective coating to chewing gum pieces prior to a hard panning process to lay down a candy coating (Dave et al. 1992). Normally only hard chewing gums can be hard panned in this way but by using a hydrated lecithin coating it is possible to candy coat and then allow the lecithin to soften the chewing gum in storage prior to consumption. The emulsifier coating when dried hard forms a suitable base for syrup-based candy coatings.

11.5.2 Caramel, Fudge, and Toffee

The unique characteristic of caramel, fudge and toffee comes from the controlled heating of dairy ingredients in the presence of sugar syrup. The resulting Maillard browning products provide both characteristic color and flavor. Concentrated milk products, such as evaporated or sweetened condensed milk, are the primary dairy ingredients used in caramel and fudge manufacture. Butter is the dairy ingredient added in toffee production.

Caramel is an amorphous sugar confection containing finely dispersed fat globules held in place by a combination of aggregated proteins and the high viscosity of the amorphous sugar matrix. Fudge is essentially a grained caramel, with the dispersion of fine sugar crystals providing the "short" texture of fudge. Some commercial caramels actually contain a small amount (perhaps 5–10%) of sugar crystals to moderate the chewy texture. Toffee is essentially a glassy sugar matrix holding the dispersed fat globules.

Fat content of caramel and fudge may be between 6% and 20%, although most commonly fat content is 10–15%. In toffee, fat content is as high as 40%. The fat in caramel provides lubricity, making the candy easier to process by preventing stickiness. The fat also aids in chewing, preventing the caramel from sticking to the teeth. The partially-crystalline fat globules, in conjunction with aggregated proteins that surround each fat globule, provide the stand-up properties of caramel and help prevent cold flow. Fats also contribute to the flavor of these confections. Although milk fat typically gives the highest quality cooked dairy flavor, many commercial products are made with vegetable fats to cut costs.

The addition of emulsifier in the formulation for caramel, fudge and toffee ensures adequate breakdown of the fat into small, well-dispersed fat globules during manufacture. Many commercial caramels are homogenized, breaking down the fat globules under pressure. The presence of emulsifier helps reduce interfacial tension of the fat droplet, allowing them to be broken down into smaller globules. The natu-

ral emulsifying properties of milk also contribute to breakdown of the fat globule emulsion. Emulsifiers also can affect the oil droplets interface−matrix interactions; by competitive adsorption and replacement of the proteins at the interface, the emulsifiers can modify the rheology of the toffees and caramels. Emulsifiers also help against coalescence of the dispersed fat globules, particularly during processing. It is not an uncommon sight to see a layer of fat forming on a batch of toffee during cooking as some of the emulsion breaks. Sometimes, further shearing can fold this separated fat back into the mass, with addition of a little more lecithin providing enhanced emulsification. Once the candy has solidified, the solid-like characteristics of the continuous sugar matrix provide the main stabilization mechanism.

The most common emulsifier, by far, found in caramel, fudge and toffee is lecithin. A common usage level is about 0.25–0.5% of the batch weight. Monoglycerides (e.g., glycerol monostearate) and diglycerides may also be used in these confections, usually at slightly higher levels (1–2%). Mono- and diglycerides are often used in low-fat confections to improve lubricity and mouthfeel.

11.5.3 Starch Candies

Starch is used as a texturing agent in a number of confections, including jelly candies and licorice. The gelation of starch after disruption of the starch granule provides the desired textural properties to starch-based confections, such as jelly bean centers, fruit slices, and gum drops. Numerous starch modification technologies have been used to moderate these textural properties.

Some emulsifiers and surface-active agents, such as GMS and Saturated Ethoxylated Monoglycerides or polyglycerate 60, are absorbed onto starch granules. This property can be used to modify the texture of starch-based sugar confectionery. Gel formation in starch-based jellies and gums is mainly due to amylose, the water-soluble fraction of starch. Interaction between amylose and emulsifiers creates a water-insoluble complex, and creates an irreversible textural effect. This interaction was quantified by Krog (1977) with the amylose-complexing index, or ACI. The ACI is defined as the percentage of amylose precipitated at 60 °C after 1 h and after reacting 5 mg of the emulsifier with 100 mg of amylose, See Table 11.4 for ACI values of some common emulsifiers. Perhaps because of this complexation, emulsifiers like GMS also are known to retard recrystallization of starch after gelatinization. To be an active amylose-complexing agent, an emulsifier must have a high level of saturated monoglycerides and some degree of water dispersibility. An example of the use of emulsifiers in starch-based confectionery is in the making of Turkish delight, where it is possible to use emulsifiers with high ACI values (like GMS) to avoid pastiness or cheesiness. Usage levels are typically 0.025%.

Azizi and Rao (2005) studied the pasting characteristics of various starches (wheat, corn, potato) in the presence of emulsifiers. The emulsifiers studied, added at 0.5% on a starch basis, included sodium stearoyl-2-lactylate (SSL), glycerol monostearate (GMS) , distilled GMS (DGMS) and diacetyl tartaric esters of monoglyceride (DATEM). In all cases, gelation temperature increased in the presence of

Table 11.4 Amylose-complexing index (ACI) values of some food emulsifiers

Glycerol Monostearate (85%)	87
Glycerol Monooleate (45%)	35
Mono and Diglycerides (50% monoester)	42
DATEM	49
Sorbitan Monostearate	18
Lecithin	16
Polysorbate 60	32
Acetylated Monoglycerides	0

emulsifiers. For example, addition of SSL caused gelation temperature of wheat starch to increase from 68.65 to 86.30 °C. At the same time, peak viscosity decreased and cold paste viscosity increased significantly with addition of emulsifiers. In most cases, hot paste viscosity also decreased with added emulsifier, except for potato starch, where all emulsifiers studied caused an increase in hot paste viscosity. The authors concluded that HLB and charge of the emulsifier both influenced the nature of the starch gels produced, and that a range of textural properties could be produced through choice of emulsifier and starch type. Galkin et al. (2014) studied the effects of ionic surfactants on starch gelatinization from three main sources: potato, maize and wheat. The addition of a strong ionic surfactant to cereal starches decreased the paste viscosity, whereas addition of a weak anionic surfactant increased paste viscosity for both cereal and tuber starches. The authors concluded that the particle size of the granular remnants affected the paste viscosity; smaller granular remnants caused by strong ionic surfactants resulted in reduced viscosity, whereas the increased particle size caused by weak anionic surfactants increased the paste viscosity.

Licorice is a flour-based starch confection that contains licorice extract. Fruit flavored licorice-type candies also fall into this category and make up a significantly larger market than true licorice. In these products, the starch granules are only partially pasted, or gelatinized, to yield a chewier texture than found in starch jellies. The protein in the flour also imparts some of the chewiness in the finished product. A small amount of fats may be added to some licorice products to reduce stickiness and enhance chewing properties, although distilled monoglycerides serve the same purpose (Jackson 1986) and may be used in conjunction with fats. Emulsifiers also delay hardening during shelf life. Based on the studies (Azizi and Rao 2005; Radhika and Moorthy 2008), emulsifiers may also influence gelatinization temperature and provide additional control of product texture.

11.5.4 Nougat and Chewy Candies

Nougats, fruit chews, chocolate chews and taffy-type products are lightly aerated candies often designed to have chewy texture. They often have no crystalline grain or may be lightly grained to modulate the chewy characteristics. Many of this class of products have anywhere from 3% to 10% fat added to reduce stickiness, enhance

processability and minimize candy sticking to the teeth during consumption. To enhance the effects of fat and to ensure adequate dispersion of the fat in these products, it is common to add 0.1–0.2% emulsifiers like lecithin, mono- and diglycerides and/or GMS.

Aeration of protein systems containing small amounts of fat, such as nougats, can be facilitated by the addition of triglycerol monostearate. However, liquid fat or lipophilic emulsifiers such as GMS or acetylated monoglycerides usually tend to destabilize foams and cause deaeration. If used in aerated products, lipophilic materials must be carefully blended into the aerated candy to minimize deaeration.

11.6 Processing Aids

Emulsifiers are sometimes used in very small amounts in confectionery products either to control aeration or to prevent product sticking to machinery and packaging. They can also be used to displace starch from starch-molded jellies and gums and provide a shiny attractive appearance as well as a barrier to degradation from atmospheric oxygen and moisture. Emulsifiers are also useful release agents providing barrier properties between product and molds, tables, metal, conveyor belts, utensils and machinery especially on cooling. Release agents must be food grade materials and have high stability to resist oxidation and hydrolysis. Acetylated monoglycerides are often used as release agents or as oiling and polishing agents because they form stable films on the surface of confectionery items. They have α-crystalline stability, a plastic, nongreasy texture and neutrality of flavor, color and odor. They reduce shrinkage, hardening through moisture loss, and prevent fat degradation and mold growth. They retain moisture and other desirable properties of the foodstuff and prevent contamination by moisture or dust. They are usually applied directly to the confectionery product by spraying. Melting points used are in the range of 30–46 °C. Typical applications include nuts, dried fruits and certain panned confectionery items. Lower melting point forms (10 °C) can be sprayed directly onto conveyers and molds to release goods with high sugar contents such as fondant creams and jellies.

Another release agent used often on chocolate-enrobing tunnels is a mixture of lecithin and cocoa butter. This is sprayed onto the band before the candy center is deposited to ensure clean separation of the centers from the band prior to chocolate enrobing.

11.7 Summary

Emulsifiers play a significant role in the processability and functionality of both chocolate-based and sugar-based confections. Typically, lecithin, PGPR, and mono and diglycerides are the main emulsifiers used in confections, but numerous other emulsifiers have been studied and shown effective in certain applications. Emulsifiers in confections can play many roles, including:

- *Emulsification and controlling oil separation*: emulsifiers reduce droplet size and stabilize fat droplets in products such as caramel, fudge, toffee and chewy candies;
- *Lubrication and reduced stickiness*: emulsifiers reduce stickiness of various confections (nougats, chews, caramel, etc.) during processing as well as during consumption;
- *Plasticizer and hydration agent*: in gum, emulsifiers soften gum base and enhance hydration of the bolus during chewing;
- *Viscosity control*: in chocolates and compound coatings, small amounts of emulsifiers such as lecithin and PGPR reduce yield stress and plastic viscosity and control flow properties;
- *Crystal modifier and bloom inhibitor*: primarily in compound coatings, certain emulsifiers influence fat crystallization during processing and can delay bloom formation;
- *Release agent*: liquid emulsifiers can be sprayed onto handling equipment to prevent sticking and release of candy pieces from molds.

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Chapter 12
Emulsifier Applications in Meat Products

M. Wes Schilling

12.1 Introduction

Fukawaza et al. (1961a) established that meat proteins are responsible for the structure of comminuted, emulsified meat products. Comminuted, emulsified meat products are finely chopped meat products such as frankfurters, bologna, and pate. Meat protein functionality is any inherent or process-generated property of proteins that affects physical and sensory characteristics of raw and finished meat products (Xiong and Kenney 1999). Meat protein functionality has been shown to determine the properties of further processed meat products, including emulsions (Fukawaza et al. 1961a; Acton et al. 1983; Ashgar et al. 1985; Gordon and Barbut 1992). The ability of chopped meat to form a three-dimensional gel matrix, emulsify fat, and retain natural and added water are important functional properties that make it possible to make comminuted meat products (Xiong and Kenney 1999) with acceptable product texture, integrity, physical stability, cooking yield, appearance, and palatability. Similarly, Samejima et al. (1985) reported that water-holding and binding properties are factors that determine the quality of emulsified meat products.

Gordon and Barbut (1992) conducted a study that revealed that the gel forming ability of the myofibrillar proteins was a major factor in stabilizing fat in a comminuted product. Rust (1987) reported that myofibrillar proteins serve two functions: (1) to encapsulate or emulsify fat and (2) to bind water. Sufficient myofibrillar proteins are necessary in comminuted product so that both of these functions are served. If all of the proteins are used in fat emulsification, water binding in the final product is low (Rust 1987). Myosin and actin in pre-rigor muscle and actomyosin in post-rigor muscle are the principal myofibrillar proteins and dictate protein func-

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tionality (MacFarlane et al. 1977; Acton et al. 1983). Salt and myofibrillar proteins are the two essential components in the production of emulsified meat products. Solubilization of myofibrillar proteins through the addition of salt, and finely chopping lean meat activates the proteins so that fat can be added and emulsified. Many other ingredients can be added to improve the stability of the emulsion, but the myofibrillar proteins are the predominant emulsifier that separates the fat from the continuous phase, which consists primarily of water and protein. This chapter will focus on ingredient functionality in meat emulsions, chemical properties that affect emulsion stability, and specific meat emulsion products. In addition, low-fat and low-sodium meat emulsion applications will be discussed.

12.2 Emulsions

Emulsions are a mixture of immiscible fluids in which one immiscible fluid is separated into small droplets within another fluid (Clark 2013). Common emulsions include water droplets suspended in oil (water/oil emulsions; w/o) such as margarine and mayonnaise and oil droplets suspended in water (oil/water emulsions; o/w) such as milk. Since water and oil are immiscible due to their hydrophilic and hydrophobic nature, food emulsions usually require an emulsifier(s) that forms a film around the suspended droplets and separates them from the aqueous phase in which they are immiscible. Proteins are common emulsifiers in food products because they are amphiphillic. This means that they have sections within the proteins that are both hydrophobic and hydrophilic. The hydrophobicity is due to the presence of a high percentage of non-polar amino acid side chains. Sections that are hydrophilic are due to the presence of a high percentage of polar amino acid side chains. For example, milk is an o/w emulsion in which the butterfat is emulsified into the aqueous phase that consists mostly of water through (1) homogenization to decrease the size of the fat globule from 1–10 to 0.2–2 μm and (2) the ability of the protein κ-casein to emulsify the fat through dual interactions with fat and water.

12.3 Meat Emulsions

Meat emulsions are similar to milk in that they are oil in water emulsions in which naturally occurring amphiphillic proteins emulsify the fat. The two essential ingredients that are needed to make a meat emulsion are a monovalent salt, typically NaCl, and the myofibrillar protein in the meat. Meat emulsions are two-phase systems that contain a dispersed phase of fat globules within a protein matrix that is solubilized in salt. A meat emulsion is considered a batter and not a true emulsion since the fat globules have a diameter larger than 100 μm and according to McClements (2005), a true o/w emulsion has fat globule diameters between 0.1 and 100 μm.

To begin the process of making a meat emulsion, lean meat is finely chopped with salt and water to extract the salt soluble, myofibrillar proteins. Chopping the meat homogenizes the meat tissue and breaks open the myofibrils to release the myofibrillar proteins into the solution. The chloride ion of the salt helps solubilize the myofibrillar proteins by increasing the overall negative charge of the protein, which helps unfold the protein, shifts the isoelectric point and increases water-holding capacity. When unfolded, the hydrophilic segments of the proteins are exposed to the water phase of the mixture. Once the proteins have been solubilized and unfolded, ground fat is then mixed with the lean meat. The hydrophobic portions of the proteins unfold and orient themselves so that they form a protein film around the fat. Once the proteins have coated the fat, the product is protected from the formation of fat caps and pockets (Pearson and Gillett 1996). After the fat has been chopped into the lean meat, the meat batter is commonly stuffed into a cellulose casing and heated to form a three-dimensional gel matrix that entraps and binds water and stabilizes the emulsion. Common meat emulsion products are frankfurters, bologna, chicken nuggets, chicken patties, and pate. Pate is a spreadable emulsion product that differs from these other products in that it is a semisolid spreadable product instead of a gel such as the sliceable products that include frankfurters and bologna (Ugalde-Benitez 2012).

12.4 Meat Proteins

Proteins within the muscle are generally classified into three groups: myofibrillar, sarcoplasmic, and stromal (Acton et al. 1983). Myofibrillar proteins constitute between 50% and 55% of the total protein content, while the sarcoplasmic proteins account for approximately 30–34% of the total protein, with stromal proteins making up the rest.

12.4.1 Myofibrillar Proteins

Myofibrillar proteins have the best emulsifying properties out of the three protein groups due to their high solubility in the presence of monovalent chloride salt and their unique ability to interact with both hydrophilic polar components and hydrophobic nonpolar components. This ability is due to the sequence of amino acids in myofibrillar proteins that have regions that are highly hydrophobic and regions that are hydrophilic in nature. Myosin and actin make up approximately 45% and 20% of the total myofibrillar proteins. These proteins are amphiphillic and able to emulsify fats once they are salt solubilized due to their water-holding capacity, viscosity, density, stability, and oil-binding ability. There are three predominant roles of myofibrillar proteins in emulsion meat products. First, these proteins are responsible for emulsifying the fat. Second, after the fat has been emulsified by the proteins, there

must be enough functional myofibrillar protein left to bind and entrap water. At this point, the overall chemical nature of the batter is hydrophilic. Third, when heated to above 60 °C, hydrophobic interactions are established through gelation and the formation of disulfide bonds. At this point, the emulsion is stable, and a protein matrix has formed through gelation that entraps water, the emulsion, and other proteins (Schmidt 1987).

12.4.2 Myosin and Actomyosin

Fukawaza et al. (1961a, b) studied the effects of myofibrillar proteins on binding in sausage. These were the earliest published results that identified myosin as the most important protein responsible for binding strength in emulsified meat products. MacFarlane et al. (1977) compared myosin, actomyosin, and sarcoplasmic proteins as binding agents in meat products. It was determined that the binding strength of myosin was superior to that of actomyosin in salt solutions up to 1 M. The binding strength of sarcoplasmic proteins was too low to be measured. These results inferred that pre-rigor muscle has greater protein functionality than post-rigor muscle. These are very important historical results because it is the initial basis for using pre-rigor meat in the production of sausage products. Gordon and Barbut (1992) stated that myosin appears to act as an emulsifier even in its native state and formed a film of defined viscoelastic and mechanical properties at the oil-water interface. Yasui et al. (1980) studied the heat-induced gelation of myosin in the presence of actin. It was determined that a specific myosin to actin ratio was essential in developing a stronger gel than formed by myosin alone. The maximum strength was observed at a myosin:actin ratio (filamentous) of 2.7, which corresponds to the weight ratio of myosin to actin of 15. Since myosin has greater emulsifying capacity than actomyosin, sodium tripolyphosphate (STP) is often added to the formulation in emulsion products. STP has three predominant functions. First, it has an overall negative charge, which helps unfold myofibrillar proteins. Second, due to its alkalinity, it increases product pH so that it is further away from the protein's isoelectric point, which improves water holding capacity. Third, STP also forms diphosphate, which disassociates actomyosin to myosin and actin. This also improves emulsifying capacity and water binding.

12.4.3 Pre-rigor and Post-rigor Meat

Pre-rigor meat is meat that has been removed from the carcass prior to chilling and rigor mortis (Sebranek 2009). At that time, pre-rigor meat can either be used to make sausage or have salt added to it to keep the meat in the pre-rigor state. Pre-rigor meat is characterized by a pH between 6.4 and 7.0 and enhanced protein solubility when compared to post-rigor meat. For optimum quality of cooked, emulsified

sausage, pre-rigor meat should be used since it provides superior emulsification ability, water-holding capacity, and cured color when compared to post-rigor meat. Pre-rigor meat protein functionality is better than post-rigor meat since the muscle has active enzymes and myosin in comparison to post-rigor meat, which does not have enzymes and contains actomyosin. In the United States, the majority of pre-rigor meat is used in fresh sausage since it gives a much firmer texture and bite than post-rigor meat and is able to maintain fresh red color under lights much longer than post-rigor meat, which is predominantly due to metmyoglobin reductase, other enzymes, and a higher pH. Since pre-rigor meat may not be readily available for the production of emulsified sausages, post-rigor meat needs to be used soon after slaughter, should not be frozen, and pre-blended to maximize emulsification capacity. Post-rigor meat is meat that is removed from the carcass after the animal has gone through the rigor mortis process. Use of post-rigor meat is inevitable in the production of emulsion sausages. However, as long as there is sufficient myofibrillar protein available to emulsify fat, bind water, and bind other protein, post-rigor meat can be successfully used to make high quality meat emulsions, including frankfurters and bologna.

12.4.4 Sarcoplasmic Proteins

The sarcoplasmic protein fraction are also referred to as water soluble proteins. There are many sarcoplasmic proteins in meat, but the two predominant ones that are important in meat chemistry are myoglobin and hemoglobin. The majority of hemoglobin is removed when the blood is removed during slaughter, but a small amount of this protein will remain in the muscle. It binds oxygen and its main function is to transport oxygen in the blood to all the cells of the body. Myoglobin makes minimal contributions to emulsification and water-holding capacity, but it does contribute to color of the final product and allows for the formation of cured color pigmentation, which also inhibits oxidation and warmed over flavor. The sarcoplasmic fraction of pre-rigor meat also contains metmyoglobin reductase, cofactors, NADH and cytochrome C which also help stabilize color.

12.4.5 Myoglobin

Myoglobin is responsible for 50–80% of meat pigmentation, depending on the muscle (Fox 1987). In fresh meat, the iron atom's sixth binding site on the porphyrin ring determines meat color. The iron atom at the center of the porphyrin ring can either exist in a reduced state, Fe^{2+} or an oxidized state, Fe^{3+}. Deoxymyoglobin exhibits a purple color in the reduced state.

Oxymyoglobin is a bright cherry red color in the reduced state, and metmyoglobin is in the oxidized state and has a brown color. Meat below the surface exists as

deoxymyoglobin since it has not been exposed to oxygen. Metmyoglobin forms either in relatively low partial pressures of oxygen or when meat has been exposed to oxygen for long periods of time and is beginning to spoil. Myoglobin is also responsible for color intensity in processed meats and cured meats. In uncured emulsion products such as chicken nuggets and chicken patties, the color intensity is dictated by the cut of meat. For example, thigh meat is often not used in these products since it has a high concentration of red muscle fibers and myoglobin and appears redder then product made from breast meat. In cured products, the porphyrin rings containing Fe in the myoglobin molecules react with nitric oxide (NO) at the sixth position to form nitric oxide myoglobin. The reaction occurs through the utilization of sodium nitrate ($NaNO_3$) or sodium nitrite ($NaNO_2$) that is dissolved in the brine solution when curing a product. Both chemicals are highly soluble in water. Sodium nitrate is utilized when one wants to slowly cure a product such as in country hams, pepperoni, or salami, and sodium nitrite is used in quick cured products such as frankfurters and bologna. In the presence of heat, nitric oxide myoglobin forms nitrosylhemochrome, which exhibits a stable pink color.

12.4.6 Stromal Protein

Stromal proteins include collagen and elastin. These are structural proteins that are found in bone, tendon, cartilage, and skin. They are important to the structure and function of muscles in the live animals. Elastin is generally not included in emulsion products, but collagen is present at levels less than 2%. Greater concentrations than that can lead to an imbalance in the ratio of collagen to myosin. If this ratio is too high, collagen will cover some of the fat particles and will appear to emulsify the fat. However, when the emulsion is heated, the collagen converts to gelatin and shrinks. This leads to an uncoated fat particle and gelatin. This phenomenon is referred to as “short meat”. Kenney et al. (1992) reported on the effects of connective tissue and gelatin on the properties of low-salt, low-fat, restructured beef. Raw and preheated connective tissue were useful for increasing tensile strength when added as 10% of the formulation. When used as 5% of the formulation, only raw connective tissue was effective for increasing tensile strength. Comparatively, Jones (1984) illustrated that the addition of collagen can improve yields and increase the firmness of a batter-type product, but a large amount of collagen can reduce stability, causing product defects such as fatting out and gel pockets. Collagen products are currently available on the market that can be added to emulsion type products to increase water-holding capacity, improve protein bind, decrease formulation costs, and increase total protein content. These products can be added at approximately 2%, and are generally produced from the same species as the meat raw material to which it is added.

12.5 Muscle Lipids

Muscle lipids are an important part of emulsified sausages because they provide desirable product flavor and a desirable texture. For beef and pork, subcutaneous fat is preferred for emulsion meat products since it predominantly consists of triglycerides that contain palmitic and stearic acids, which contribute to fat hardness, and oleic and linoleic acids, which contribute to softness (Hogenkamp 2011). Fats that are more highly saturated, such as those from beef, are harder to stabilize. Fats from pork and chicken contain higher concentrations of unsaturated fatty acids, making them softer and easier to stabilize. The variations in fatty acid profiles warrant the use of different processing conditions depending on the specific product being processed. Optimum emulsion temperatures for beef, pork, and chicken are >18, 14, and 4–8 °C, respectively. Chopping an all-beef meat batter to approximately 18–20 °C, after the addition of fat and then stuffing into cellulose casings at this temperature will produce an all-beef frankfurter. If the temperature is too low, the fat will be too solid for the protein to be able to emulsify the fat. All pork frankfurters should be chopped to a temperature of approximately 14–16 °C so that the fat will liquefy and the protein can emulsify the lipid. Hogenkamp (2011) stated that for frankfurters that are made from mechanically deboned meats, the final emulsification temperatures for poultry, pork and beef should be 8–10, 12–14, and 18–20, respectively. Colder temperatures prevent the disruption of the fat cell walls and inhibit the protein's ability to emulsify the fat since it is so hard.

12.6 Factors Affecting Protein Functionality

12.6.1 Salt Type

Sodium chloride, or table salt, is the most common nonmeat adjunct added to further processed products. It contributes flavor, preserves the product, and solubilizes myofibrillar proteins (Rust 1987). The ability of salt to solubilize the myofibrillar proteins is of vital importance to the successful manufacture of emulsified sausages (Rust 1987). Salt solubilizes myofibrillar proteins by increasing the electrostatic repulsion between the filaments and alleviates some of the structural constraints of myofibrillar proteins. Barbut and Findlay (1991) tested the effects of using different salts in the stabilization of meat batters. Results suggested that divalent cations such as Mg^{2+} and Ca^{2+} destabilized the meat batters. Mg^{2+} caused extensive precooking and protein matrix aggregation and poor fat stabilization because of insufficient protein film formation. Calcium ions caused widespread protein aggregation during cooking, which led to extensive fat and water losses. However, the use of NaCl and KCl to form stable meat batters was successful because they contain monovalent cations.

12.6.2 Effect of Species and Muscle Fiber Type

Xiong and Brekke (1991) determined that fast-twitch (breast) and slow-twitch (legs) myofibrils in chicken muscle are affected differently by rigor state, pH, and heating properties. Muscle fiber type affected protein extractability and gelation properties of myofibrils. Post-rigor breast myofibrils demonstrated greater protein extractability and gel strength than pre-rigor breast myofibrils, but the reverse was found for leg myofibrils. In addition, Ugalde-Benitez (2012) stated that gels that were made with chicken breast formed stronger, more stable gels than muscles with predominantly red muscle fibers, regardless of pH and ionic strength. The optimum pH for gelation of chicken breast and leg myofibrils were 6.0 and 5.5, respectively. In comparison, a pH value of 6.0 is considered optimal for gelation in pork and beef species (Yasui et al. 1980). The reverse was true in leg myofibrils. It was concluded that muscle rigor state had more effect on protein extractability and gel strength for breast myofibrils than leg myofibrils. Petracci and Bianchi (2012) also reported that legs and drumsticks cause harder texture, darker color, higher fat content, and stronger flavor than white muscle such as breast meat. Similarly, using rabbit skeletal muscle, Boyer et al. (1996) studied differences in heat-induced gelation of myofibrillar proteins and myosin from fast and slow-twitch rabbit muscles. Proteins from slow-twitch muscle exhibited higher thermostability and lower gel strength than proteins from fast-twitch muscle. Slow twitch myosin's gelling ability decreased in the absence of actin, but fast-twitch myosin's gelling ability increased in its presence.

12.6.3 pH and Ionic Strength

Schmidt (1987) stated that protein extractability increases with pH elevation from 5.5 to 6.0, with 6.0 being the optimum (Yasui et al. 1980; Ishioroshi et al. 1979). Schmidt (1987) also stated that the addition of 2.5% NaCl used to extract protein decreased pH by 0.1–0.2 units. Use of phosphates in a meat batter increases pH, which enhances protein extractability and offset the decrease in pH caused by NaCl (Schmidt 1987). In addition, Petracci and Bianchi (2012) stated that NaCl lowers the isoelectric point of myofibrillar proteins from approximately 5.2–5.0, which increases water-holding capacity. Samejima et al. (1985) conducted a study to predict binding in comminuted meat products by characterizing myofibrils (beef) properties with respect to gel formation and protein extractability. Ionic strength of the solution up to 0.6 M NaCl increased gel strength and the addition of pyrophosphate in 0.3 and 0.6 M NaCl ionic strength batters increased protein extractability and gel strength. Hogenkamp (2011) stated that an ionic strength of 0.5 needs to be reached in order to fully solubilize the myofibrillar proteins.

12.7 Industrial Processing of Emulsified Meats

12.7.1 Equipment and Process

There are two predominant methods to make emulsified sausages in the food industry. The first method is a batch process in which it is easier to control the quality of the product. The second method is a continuous process that makes it possible to maximize production.

12.7.1.1 Batch Process

12.7.1.1.1 Grinding, Emulsifying, Stuffing and Linking

A commercial meat grinder is used to grind lean meat through either a 1 or 5/8 cm grinder plate. The fat portion or carcass trimmings are then ground through a 1 cm grinder plate. For example, if this is all beef frankfurters, the lean beef would be ground through a 5/8 cm grinder plate and beef fat would commonly be ground through a 1 cm plate.

After grinding, the meat will either be added to a chopper or an emulsifier. If a chopper is used, the lean meat is placed in the chopper with a brine solution of water, phosphate, salt, sodium nitrite, sugar, erythorbate, and spices. The phosphate should always be dissolved in the water first using a tank mixer prior to adding the other ingredients. After chopping under vacuum, the fat portion of the meat should be added and mixed further. This mixture needs to be chopped until approximately 18–20 °C for the emulsion to form since that is the temperature that beef fat is liquid enough for the myofibrillar proteins to coat the beef fat. After 18–20 °C, the product is ready to be stuffed into the meat casings. Often for optimum texture and chew, meat processors will use a cutter or chopper with a colloid mill to homogenize the emulsion prior to stuffing. An emulsifier can also be used instead of a chopper. An emulsifier combines the functions of grinding and chopping in one piece of equipment and allows more rapid production of emulsion products (Pearson and Gillett 1996). It is called an emulsifier because it takes uniform meat raw material and uses a rotary blade and grinder plate in tandem as one device so that the protein encapsulates the fat and an emulsion is produced. A common application for an emulsifier is in the production of frankfurters that are made from poultry or frankfurters formulated from mechanically deboned chicken and turkey and chicken and pork fat. The mechanically deboned meat is often frozen. The mechanically deboned meat, brine and turkey meat are mixed to uniformity and then placed in the emulsifier at a rate of 3000–5000 lb/min. The temperature of this batter should be 10–14 °C so that both the poultry fat and pork fat are liquid enough to be emulsified. After passing through the vacuum chopper or emulsifier, the product is ready to go to the stuffer. A vacuum stuffer is used that is either a piston stuffer or a combination of a piston and a pump. It has a horn that is placed on the stuffer that varies in diameter

depending on the diameter of the sausage. Frankfurters are commonly stuffed into casings with diameters of 25–32 mm. Vacuum is important in the stuffer to prevent air pockets in the product. After stuffing, automated linkers are used to form links such that 10 links per pound are made. Frankfurters are commonly clipped with a hanging tie and suspended from a hook so that they hang vertically in a smokehouse. Larger diameter products such as bologna are commonly placed horizontal on a screen. Many meat processors still use batch processing and prefer it because they can make changes in formulation during the process and have more control over finished product quality.

12.7.1.1.2 Smoking and Cooking

A smokehouse is used to heat and stabilize the emulsion through protein coagulation. When emulsified sausages have been placed in casings and introduced to the smokehouse, they generally have internal temperatures between 15 and 21 °C. These internal temperatures are usually increased to 71 °C in a 4–5 stage process. The smokehouse is usually set at approximately 55 °C with the damper open for a time ranging from 10 to 30 min depending on the diameter of the product. This allows the sausage surface to dry so that smoke can be applied. After this, the damper is usually closed to increase the relative humidity and the product is heated at approximately 68 °C. At this point, smoke can be imparted if desired. After this step, the product is heated to temperatures ranging from 74 to 85 °C. Temperatures higher than this can lead to fatting out, peeling problems, and other quality problems. In addition, the relative humidity should generally stay between 35% and 40%. Relative humidity greater than 45% can lead to fatting out in beef emulsions (Pearson and Gillett 1996) because high moisture contents in the atmosphere can lead to excess moisture inside the moisture permeable casing which can lead to emulsion breakdown since proteins bind both water and fat.

12.7.1.1.3 Chilling

The smoked and cooked samples are generally chilled to approximately 0–2 °C and then peeled and packaged on automated production lines. Some companies use steam pasteurization or hot water dip tanks to treat the product post-packaging to help control *Listeria*. After vacuum packaging, the product generally has approximately 90 days of shelf life. It can be reheated multiple times since nitroso hemochromogen, produced through heating nitric oxide myoglobin inhibits oxidation that occurs from multiple cooking cycles. Cured meat products can be reconstituted at home or restaurants through boiling in water, direct heat through frying or grilling and heating in the microwave.

12.7.1.2 Continuous Processing Systems

Due to the demand for higher output in the production of cooked emulsion sausages, continuous coextrusion processes have been introduced to meat plants. Two of these systems include the Marel Townsend QX and the Cozzini Prime Cut. The Cozzini Prime Cut is a continuous system for emulsion production that includes grinding and chopping in one system and stuffs out a homogenous emulsion. The Marel Townsend QX system makes emulsion sausages through coextrusion technology of a continuous flow of meat batter and a layer of collagen. Two separate pumps are used; one for the batter and one for the collagen casing. Marel and Handtman also offer coextrusion technology where alginate is use with CaCl to form a gel on sausage products. The chemistry of emulsion formation is similar, if not identical to that of batch processing but allows for processors to have greater production capacity.

12.7.1.2.1 Preblending

Preblending is the grinding and mixing of meat ingredients with either some or all of the salt and sodium nitrite prior to mixing together all of the components in the formulation. This is commonly done in the meat industry in a number of applications. It is also utilized in conjunction with least cost formulation software to minimize ingredient costs (Pearson and Gillett 1996). Preblending helps control spoilage, allows the use of preblended meats in the formulation of multiple products, helps control product composition, inhibits lipid oxidation due to the presence of sodium nitrite, speeds the curing reaction, and can be combined with least cost formulation software to optimize the formulation. Preblending with salt and nitrite will increase the shelf life of the raw meat material from 2–3 days to approximately 1 week. This is very important since an emulsion product may be made at a different facility from where the animals were harvested. Most pre-rigor meat is pre-blended immediately after slaughter and deboning. This prolongs the shelf life of the raw material and maximizes emulsifying capacity and is commonly done for bull meat and sow meat. Preblending prevents oxidation because the sodium nitrite reduces to nitric oxide in the presence of ascorbate or erythorbate. Nitrite binds nonheme iron as well as the iron molecule in myoglobin to prevent iron from catalyzing oxidation in the fresh raw meat material and the final, cooked emulsified product, and allows it to be reheated multiple times without the occurrence of warmed-over-flavor (Claus et al. 1994). Preblending also streamlines the process since the meat is allowed to cure in transit either to other plants or from the boning room to the area where further processing occurs. This allows for more efficient use of processing equipment used to make emulsion sausages such as mixers, choppers, and smokehouses.

12.7.1.2.2 Least Cost Formulation

For sausage production, preblending facilitates the use of least cost formulation software in the production of sausages. These two technologies can be used separately, but they are most often used together to receive maximum benefit. For example, after preblending, meat blocks are commonly evaluated for fat, protein and moisture percentage using either Near Infrared (NIR) or Mid Infrared Spectroscopy (MIR). If a processor is targeting 25% fat in a frankfurter, two meat blocks could be used that may have 15% and 35% fat. Cooking loss also needs to be considered to determine the exact amount of each of these two meat blocks to target 25% fat. If the maximum value of 30% fat is desired in an emulsified product, infrared spectroscopy, preblending, and least cost formulation can be used to maximize fat percentage to minimize cost while ensuring that the product will be below 30% fat (the legal limit in the United States). Least cost formulation consists of using computerized linear programming to minimize costs using available raw materials, product cost, chemical composition, color, protein bind, connective tissue amount, and any constraints on the finished product. Based on raw material possibilities, the linear programmer will come up with a color score, flavor score, and binding score. The binding score is essential because it is a measure of whether or not the raw materials will provide enough salt soluble protein to produce a stable product emulsion. An in-depth explanation on the principles of least cost formulation can be found in Pearson and Gillett (1996).

12.8 Role of Nonmeat Ingredients

12.8.1 Salt

Salt in the form of sodium chloride is the most important nonmeat ingredient in emulsified meat products. Without the chloride ion, it would be impossible to solubilize the myofibrillar proteins and activate them so that they are able to bind water, entrap fat and exhibit acceptable texture. Sodium chloride is introduced into the emulsion in a brine solution that also includes phosphate, sodium nitrite, ascorbate, erythorbate, and other ingredients. In addition, salt also imparts a desirable salty flavor, enhancing the overall product flavor, lowers the water activity and slows microbial growth. The solubilization of the myofibrillar proteins through the addition of sodium chloride allows the protein to unfold and encapsulate the fat, which leads to the formation of the meat emulsion.

12.8.2 Alternative Salts

Sea salt is a broad term that generally refers to unrefined salt derived directly from the ocean or sea and is not refined to the level of most commercial salts. It contains traces of other minerals including iron, magnesium, calcium, potassium, manganese,

zinc, and iodine. Table salt, on the other hand, is the most common salt found in the average kitchen, as well as the industry. Table salt usually comes from salt mines and is refined to the point that minerals are removed until it is pure sodium chloride. The industry continues to develop new products in which sea salt is substituted for pure sodium chloride. Sea salt has a lower sodium content than refined sodium chloride because of the innate impurity of the unrefined salt. Sea salt is gaining popularity in the industry because it allows for yields that are equivalent to products that are made with traditional salts while lowering sodium levels and maintaining the desired flavor. Practically, the use of these salts will only lower the sodium content by approximately 10% since NaCl is still the predominant component of the sea salt.

Researchers have reported that using salt in a flaked and/or an agglomerated form improves the protein functionality and taste bioavailability of sodium when compared with granular salt (Desmond 2006). The agglomerate structure gives the salt a sponge-like texture, with an increased surface area that enhances its solubility, ability to extract protein, and flavor development. This salt is more bioavailable, thus able to improve water and fat binding. This increases the ability of the meat system to release volatile compounds into the atmosphere, for the product to retain more fat, and the ability of protein receptors to perceive salt. All of these mechanisms improve flavor, thus allowing for slightly lower concentrations of sodium chloride in the finished product. Another concern for the industry is food safety in regards to microbial growth. By reducing the sodium level, there may be an adverse effect on shelf life. Salt has always been the most complete preservative in the industry's quest to extend the shelf life of meat products.

12.8.3 Phosphates

It has been known for over 50 years that phosphates increase the yields of muscle food products (Bendell 1954). Sodium and potassium salts of phosphoric acid are the foundation for any nonmeat ingredient formulation. While most ingredients directly manage added water in the formulation, phosphates manage the muscle food proteins and thus the water already inherent in the meat tissue plus additional formula water. The proper inclusion of phosphates can have many positive effects on the muscle food system and final product including (1) functioning as an antioxidant by tying up metal ions and preventing them from initiating oxidation reactions; (2) improving myofibrillar protein extraction through increasing ionic strength and separating actin and myosin bonds to form actin and myosin; (3) increasing pH, which leads to greater water-holding capacity; and (4) functioning as a buffer and resisting pH change when acids are added to the meat product.

Phosphates are typically manufactured by three primary drying methods: drum drying, spray drying, and agglomeration. Of these three methods, agglomerated phosphate products have the greatest solubility (Brouillette 2007). Benefits of improved solubility include faster marinade and pickle formulation, less sensitivity

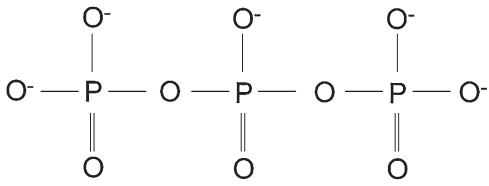

Fig. 12.1 Representation of the structure of tripoly phosphate

to solubility in cold water, increased salt tolerance, and improved yields and performance in hard water systems.

Food grade phosphates are manufactured through the reaction of the raw phosphoric acid with either sodium or potassium hydroxide yielding a salt of phosphoric acid. This process allows phosphate components to vary in size, chain length, and therefore specific actions in the meat. A basic phosphate product, commonly known as tripoly phosphate is a three phosphate chain compound. A representation of this is seen in Fig. 12.1.

The other various chain length compounds of phosphates would be similar structures with specific numbers of phosphorous components designated by the "P" and a corresponding number of oxygen molecules—"O". A monophosphate has one phosphorous, di- or pyrophosphate has two phosphorous units, tri polyphosphate has three phosphate units and polyphosphates have four or more phosphate units. Monophosphates are important components in acidic marinades since they buffer pH. Di or pyrophosphates extract muscle proteins, separate actomyosin into actin and myosin, and bind Mg^{2+} in hard water. Tripolyphosphates bind calcium and easily break down to the diphosphate form in solution, and polyphosphates bind Ca^{2+} and improve phosphate solubility. Use of a blend of phosphates with multiple chain lengths often can maximize functionality in a processed meat product and should be considered for use if it is cost effective.

12.8.4 Sodium Nitrite

Sodium nitrite is used in frankfurters and other emulsified products at levels as high as 156 ppm. The nitrite reduces to nitric oxide and then binds with myoglobin at the iron's sixth binding site. Sodium erythorbate or an ascorbate are added to provide an electron source that functions as a reductant in the reaction forming nitric oxide from nitrite (Claus et al. 1994). This adjunct is necessary since nitrite is a strong oxidizer and cannot be reduced without the assistance of a cure color accelerator. Nitrous acid is one of many intermediates that can form in the cured color reaction that permits the reduction to occur that causes the formation of nitric oxide (Fox 1987; Aberle et al. 2001). Nitric oxide is a volatile gas that forms an unstable compound by binding with myoglobin to form nitric oxide myoglobin. Nitric oxide myoglobin is stabilized into nitrosylhemochrome when the meat product is heated. Nitrosylhemochrome is a desirable pink pigment that prevents warmed over flavor,

extends shelf life, inhibits *Clostridium botulinum* growth, and provides desirable flavor (Aberle et al. 2001). Nitrite is limited to 156 ppm in comminuted or sectioned and formed products since nitrosamines, a carcinogenic substance, can be formed from residual nitrite when heated in a meat product (Claus et al. 1994).

12.8.5 Soy Protein

Soy proteins are utilized in processed meat products as soy flour, soy protein concentrates, and soy protein isolates (Pearson and Gillett 1996). The impetus for use of soy proteins is that they provide more desirable functionalities in meat applications than more expensive animal source alternatives such as casein or dried milk. Soy proteins provide water absorption and binding, gelation, cohesion-adhesion, emulsification, and fat absorption when formulated in processed meats (Fulmer 1995). Soy protein can be incorporated into meat products at levels up to 3.5% of the finished product in frankfurters, deli hams, and other processed products (Pearson and Gillett 1996). Soy protein concentrates contain at least 70% protein, have less off flavor than soy flour, but are more expensive. These proteins are ideal for utilization in restructured meats for two reasons. First, they are relatively inexpensive when compared to meat and milk proteins.

Second, soy protein concentrates have special gelling properties that aid in binding chunks of meat together (Hermansson 1986; Pearson and Gillett 1996) and enhance water and fat binding.

Soy protein isolate is made up of at least 90% protein. It has practically no off-flavor, with excellent gelation, water binding, and fat binding characteristics. Its functionality is superior to that of soy protein concentrate, but it is not as practical to use since the increase in cost outweighs the increase in functionality. Hermansson (1986) reported that soy protein gelation due to heating occurs by association of molecules into strands in an ordered arrangement. This provides good functionality for protein and fat interactions in processed products ranging from emulsions to sectioned and formed products.

12.8.6 Modified Food Starch

There are four primary factors that must be considered when selecting a starch for use in emulsified meats. First, the native starch must be modified (usually less than 0.1% modification) at the hydroxyl groups so that it provides the desired improvements in product quality. When a native starch is heated in the presence of water, it will swell, which allows it to trap and bind water. When the product is cooled, the starch molecules will associate with each other and push the water out of the food product, through a process known as retrogradation. Modification of the starch molecules will prevent the starch molecules from reassociating. The second important

factor to consider is the gelatinization temperature of the starch. This temperature must be lower than the final internal temperature that the product is cooked to so that the starch is able to gelatinize and maximize yields. Two additional characteristics of modified food starches that are important include pasting temperature (temperature at which hydration and viscosity begins to develop) and temperature of peak viscosity (temperature at which the starch granules are fully hydrated and demonstrate maximum viscosity). Modification (cross linking) of starches also influences the pasting temperature. A critical decision in starch selection is to be sure the pasting temperature and temperature of peak viscosity fall within the processing temperature range of the product and process in development.

The type of modification that a starch undergoes is important, and most often depends on its function within the food product (Schilling and Smith 2010a). Starches can be modified through either cross-linking or stabilization. Cross-linked starches are commonly used in processed meat products since they prevent retrogradation and improve yields. Cross-linked starches increase resistance to shear and low pH conditions, and produce pastes with greater viscosities than native starches. Stabilized starches are sometimes more functional than cross-linked starches because they lower gelatinization temperature and pasting temperature and prevent water from leaving the food product. Starches can also be both cross-linked and stabilized to maximize functionality. Modified food starches such as cross-linked starches will increase yields in emulsified products such as frankfurters or bologna when used at percentages up to 2–3% of the finished product. When formulating low-fat frankfurters, modified food starch must be used, along with ingredients to mimic fat properties. This will ensure that enough water is bound so that the product has acceptable sensory properties.

Modified food starches can be used in poultry meat or sauces within entrees to improve product yields and maintain quality. If these products are frozen, a substitution needs be made on the starch that provides freeze-thaw stability such as acetylation, hydroxypropylation, or phosphorylation. Use of the appropriate modified starches in entrée products will improve yields and enhance product quality. Starches can also be used as coatings in meat products that can adhere seasonings, flavor and colorants to poultry products. In addition, there are many novel starch products that have been released on the market that hydrate in cold or hot water and provide freeze-thaw stability.

12.8.7 Carrageenan

Commercial carrageenans can be utilized in emulsified meats and other processed meats to improve product quality (Schilling and Smith 2010b). The three types of carrageenans that are available for use in the food industry include kappa, iota, and lambda. Commercial blends of kappa and iota are commonly utilized in further processed poultry products to maximize yields and optimize texture.

Important factors with respect to carrageenan use include proper dispersion, concentration of carrageenan in the product, functional properties of the carrageenan form, and proper methodology for incorporating carrageenan into the product. The most challenging part of using carrageenan is successful dispersion in the brine solution and/or meat product. Carrageenan is usually the last ingredient that is added to cold temperature brines and needs to be incorporated with a cation (salt, calcium, etc.) or carbohydrate (dextrose, maltodextrin, etc.) carrier. Carrageenan needs to be incorporated in cold water to minimize solubility prior to cooking. In addition, in order to keep the carrageenan dispersed in the brine, a high shear mixer with an impeller head is essential. Carrageenan should be used at concentrations of 0.2–0.6% in the finished product. Since carrageenan can bind approximately 25–40 times its weight in water, its concentration in the product can be increased as the amount of water that is added to the product increases. In general, if more than 0.6% carrageenan is added to the product, there will be no further improvement in yields because there is not enough water present to hydrate the carrageenan. Using too much carrageenan or improperly dispersing carrageenan can also lead to product defects including rubbery, gel-like textures, gel pockets, and tiger striping.

Commercial carrageenans do not bind water and form a gel until the product is heated above 140 °F, which solubilizes the carrageenan. After heating, the product is cooled and the carrageenan forms a gel matrix that traps water. Therefore, carrageenans provide the most functionality in deli type meats that are consumed cold and other pre-cooked products. When included in formulations for frozen products, special care must be given to carrageenan selection. A carrageenan blend containing a portion of iota carrageenan will provide freeze/thaw stability, while kappa carrageenan will yield the stronger gel desired for product firmness.

In emulsified products, carrageenan can be added directly to a bowl chopper prior to protein extraction (formation of the emulsion) or after protein extraction as a dispersion in water. Kappa carrageenan can be used in low-fat emulsified products such as chicken frankfurters to increase yields and mimic fat properties. Its functionality will be enhanced if used with starch at about a 4:1 ratio (2% starch, 0.5% carrageenan) and konjac flour or xanthan gum to help mimic fat properties.

12.8.8 Sodium Caseinate

Sodium caseinate is another nonmeat additive that is commonly used in emulsified meat products, since it can help stabilize the product when exposed to high temperatures. Acidification, as in cheese making, separates the casein from the rest of the skim milk product by decreasing the pH of the milk to the isoelectric point of casein (Walstra et al. 1999).

Acidification produces a casein gel product that can be separated by either centrifugation or through a vibrating sieve. The acid casein produced is at its isoelectric

point making it insoluble in water and alkaline solutions. Insolubility gives acid casein no significant functionality in processed meats. The molecular structure has not been denatured, and the product is pure (>90% protein). Taking this acid casein and diluting it with an alkali solution such as sodium hydroxide purifies it and increases its solubility. Titrating acid casein to between 6.6 and 7.0 gives the greatest solubility of sodium caseinate and allows for its best water binding and emulsifying properties (Swaisgood 1996). Su et al. (2000) utilized 2% sodium caseinate in frankfurters and revealed that it increases shear force and thermal stability. Atughonu et al. (1998) demonstrated that 2% caseinate increased protein content, cooking yield, and decreased fat content. Electron micrographs demonstrated that sodium caseinate was able to bind to meat protein forming a protein-fat matrix with less coalescence of fat droplets. One specific application of sodium caseinate in meat products in which the fat is exposed to high temperatures and pressures is in the production of canned luncheon meat and frankfurters (Hogenkamp 2011). Sodium caseinate is the best ingredient to stabilize the fat and retain the firmness of texture in these applications.

12.8.9 Fibers

Many commercially available fibers are available for use in processed meat products and include a mixture of cellulose and other fibers such as pectin, lignin, and hemicelluloses. Fibers that contain a greater amount of pectin generally have greater water binding than other fibers. For example, citrus fiber is an excellent water binder but does not bind fat well. Oat fiber has been utilized in light bologna and fat-free frankfurters at 3% to increase yield and decrease purge loss, but resulted in product with less red color, and increased hardness (Steenblock et al. 2001). Oat bran/fiber offers unique merits including mimicking fat texture, absence of cereal flavor, and retention of natural meat flavor. However, the critical element for fiber-added meat products is to make sure that it still meets the sensory expectation of the consumers, since this is more of a market driver than potential yield and texture improvements or health benefits.

12.8.9.1 Water Binding Capacity/Moisture Retention

The primary functional role of dietary fibers in processed meat products is to bind water, which leads to reduced cooking loss and increased yield. For example, carboxymethyl cellulose forms a gel upon heating, which entraps water thus making it suitable for use in emulsified products. Cellulose derivatives such as microcrystalline cellulose (MCC) are found in meat products such as chicken nuggets, patties, and turkey sausages and can be used as a flavor carrier, emulsion stabilizer, and/or to improve freeze-thaw stability (Codex General Standard for Food Additives 2011).

USDA limits the amount of cellulose to 3.5% in nonstandardized comminuted poultry products (USDA-FSIS 2007). Sodium in the form of sodium chloride or phosphate can be reduced in a formulation through fiber addition. Fruit and vegetable fibers can be used to retain the increased amount of water that is often used in the production of low-fat products. In addition, oat fiber mimics fat texture and binds water in low-fat products and can be used to replace animal fat with polyunsaturated vegetable oil. For example, JRS Company formulated a 65% fat reduced sausage by including oat fiber, MCC, and inulin, in the formulation (Bodner and Sieg 2009). These fibers made up a total of 3% of the meat block and were effective at binding water, mimicking fat, and preventing oil from leaching out of the cooked product. In this application, mechanical shear activates gel formation by the MCC and inulin which leads to water and fat retention. Dietary fibers can be used to reduce oil uptake. Fibrex and pectin were used in batter/breading and decreased oil uptake (up to 30%) in fried chicken, while increasing water retention and consequently improving texture (Karlovic et al. 2009). Fibrex is a gluten-free commercial product derived from sugar beets and predominantly contains cellulose, hemicellulose and citrus pectins and increases product stability during frying and freezing. Dietary fiber also offers nutritive benefits. For example, inulin is a prebiotic and fruit fibers such as grape and citrus contribute antioxidant capacity. These fibers are gaining popularity in Europe, where prebiotics and other fibers are increasing in use in meat products.

12.9 Meat Emulsion Structure

Meat emulsions are characterized by myofibrillar proteins encapsulating the fat globules within the food matrix. Salt is used to extract the salt-soluble, myofibrillar proteins, which orient themselves to the fat globules as seen in Fig. 12.2. The protein membrane that surrounds the fat globule consists of many myofibrillar proteins, including but not limited to myosin, actin, and actomyosin which make up more than 65% of the protein and troponin, tropomyosin, desmin, nebulin, and titin. When heated, the emulsion is stabilized by the formation of protein cross-links that form between protein membranes (Fig. 12.2), predominantly due to the formation of disulfide bonds. Other components of the emulsion include myofibrils, which contain myofibrillar proteins and collagen. Ingredients such as starch and carrageenan can be used to bind water and help stabilize the protein structure. Amphiphillic proteins such as soy protein and sodium caseinate will bind water but will also orient themselves to the fat globule membrane and help stabilize the emulsion.

Scanning electron microscopy (SEM) is used to magnify and view the surface structure of meat emulsions. Fig. 12.3 shows the protein film that surrounds fat globules in a meat emulsion (Barbut 1988). The top picture demonstrates an emulsion in which an insufficient amount of salt was used. In general, 1.8–2.0% salt is needed to fully solubilize myofibrillar proteins to effectively encapsulate fat and provide a firm, uniform texture. The bottom picture in Fig. 12.3 represents the sur-

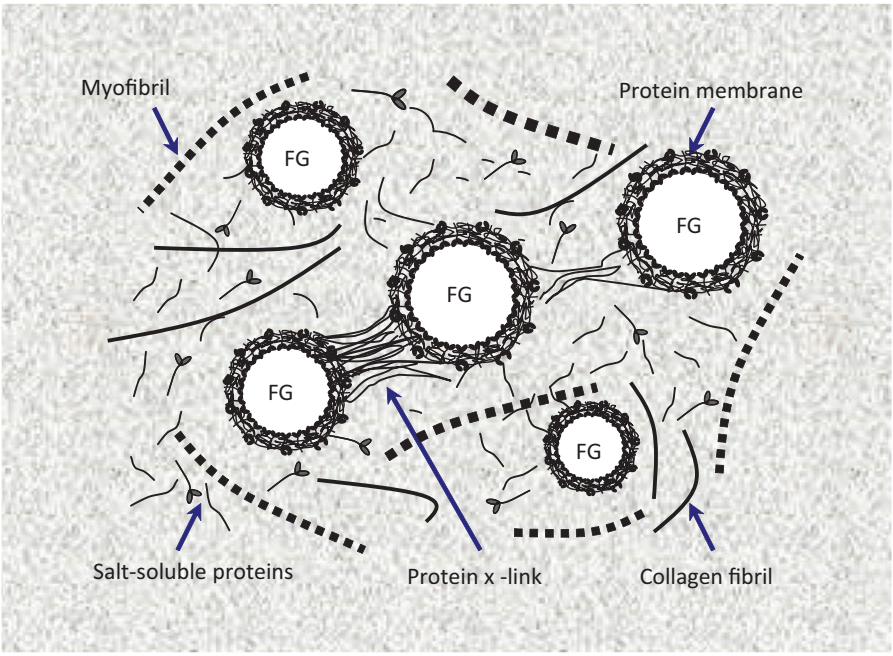

Fig. 12.2 Orientation of proteins and fat globules (FG) in a meat emulsion. (Picture courtesy of Youling Xiong, University of Kentucky)

face of a protein film surrounding a fat globule with a sufficient amount of salt in the formulation. The nonpolar portion of the salt soluble proteins orient to the fat globule while the polar portion of the myofibrillar proteins orient to the outside of the protein membrane so that it interacts with the aqueous portion of the protein matrix.

Transmission electron microscopy (TEM) can be used to view the different parts of a meat emulsion. Figure 12.4 (Gordon and Barbut 1990) shows a detailed magnification of a meat emulsion. In this figure, the fat globule is denoted as "f" and is encapsulated by the salt-soluble, myofibrillar protein. The "im" indicates the internal membrane of the fat globule that interacts with "i". "i" represents where hydrophobic, non-polar portions of the myofibrillar proteins orient to the fat globules. The "i" interconnects the diffuse region of proteins to those proteins that stabilize the emulsion at the fat globule membrane. The "e" represents where the emulsion occurs. "p" represents the membrane that surrounds the fat globule. This protein membrane is hydrophobic where it interacts with the fat globule, amphiphilic where it exists between the protein at the fat globule interface and the aqueous product matrix "m". The protein is hydrophilic where it interfaces with product matrix "m". The matrix m consists of water, collagen, water soluble proteins such as myoglobin, protein cross-links, salt-soluble protein and myofibrils.

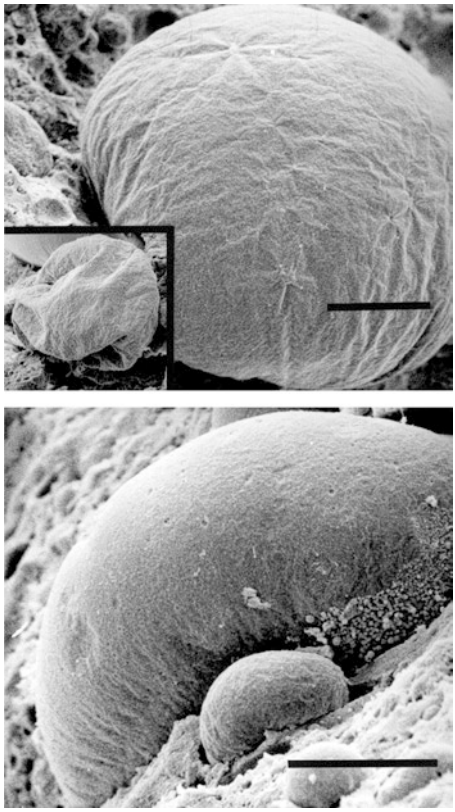

Fig. 12.3 Scanning electron micrographs of fat globules with surrounding interfacial protein film in a cooked, low salt poultry meat batter formulated with 1.5% NaCl (top), and with an adequate salt level of 2.5% NaCl (bottom). Bars = 10 μm. Reproduced from Barbut (1988). (Images courtesy of Shai Barbut, University of Guelph)

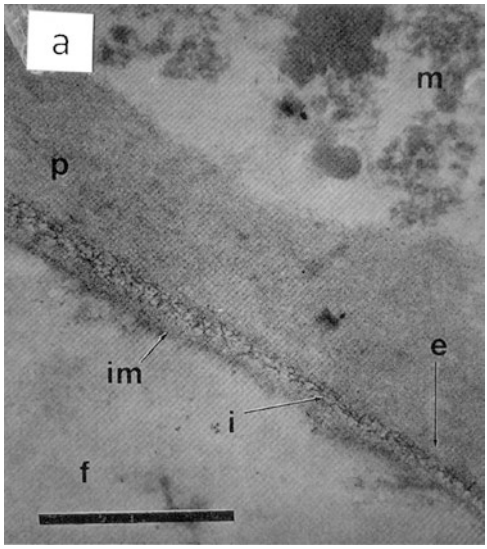

Fig. 12.4 Transmission electron micrograph of fat globules in a cooked meat batter produced with KCl (ionic strength = 0.43). Showing magnification (of the interfacial protein film surrounding the fat globules within the protein matrix of the batter. f = fat; m = matrix; p = thick diffuse protein coat; im = internal membrane; i = interconnecting diffuse region. Bar = 1 μm. (Reproduced from Gordon and Barbut (1990). (Image courtesy of Shai Barbut, University of Guelph))

12.10 Emulsified Meat Products

12.10.1 Frankfurters

12.10.1.1 Cooked Sausage

12.10.1.1.1 Cured Sausage

Frankfurters are commonly formulated with salt, sodium and/or potassium lactate, sodium diacetate, corn syrup, dextrose, sodium phosphate, sodium ascorbate, and sodium nitrite. The majority of cooked sausages include sodium nitrite and an isomer of sodium erythorbate since these ingredients along with heat form nitrosylhemachrome and prevent warmed over flavor (WOF). Other antioxidants are not generally included in cured sausage, but some spices have natural antioxidant properties. Sodium tripolyphosphate or a phosphate blend is commonly used to improve yield and quality as well as to provide some antioxidant capacity. Frankfurters and bologna can have up to 30% fat and the combination of fat and added water can be no more than 40% (CFR 9.319.180 2011). Added water is defined as the moisture percentage of the product minus 4 times the protein concentration of the product subtracted from the moisture percentage of the product. Details on the procedures and equipment for producing frankfurters are listed previously in Sect. 12.7. Bologna is very similar to frankfurters and is prepared using a similar process and formulation with the exception of stuffing into a larger diameter casing and having a much longer cooking time in the smokehouse. Frankfurters can take as short as an hour to heat process and bologna can take as long as 8 h to process in the smokehouse.

Antimicrobials are used to protect against spoilage bacteria and pathogens, predominantly *Listeria monocytogenes*. Sodium or potassium lactate and sodium diacetate are bacteriostatic to *Listeria monocytogenes* and spoilage microorganisms. These ingredients have synergistic effects and are used in many cured cooked sausages. Sodium or potassium acetate or vinegar can be used in the place of diacetate. Lemon powder can also be used since it is a source of citric acid and vitamin C (Schilling 2013).

Most cured sausages are either wood smoked or treated with liquid smoke, which imparts phenolic compounds and carboxylic acids that have both antimicrobial and antioxidant properties. In addition, pyrogallol and resorcinol are polyhydroxyphenolic compounds in smoke that function as antioxidants. One antimicrobial treatment that should be considered for cured cooked sausage is post packaging submersion in hot water or steam pasteurization since it will control any *Listeria monocytogenes* cells that could have contaminated the surface after processing but prior to packaging.

12.10.1.2 Naturally-Cured Frankfurters

Naturally cured frankfurters are formulated with sea salt, cultured dextrose, cultured celery juice or celery powder, vinegar, and cherry powder as antimicrobials, curing agents, and curing accelerators (Schilling and Williams 2014). Cultured dextrose contains propionic acid and acetic acid and is the most abundant antimicrobial in the product. Celery powder is a natural source of nitrates (up to 30,000 ppm nitrate) that are broken down into nitrites through bacteria in the meat and the lactic acid starter culture in the celery juice. Pre-converted natural curing systems such as cultured celery juice have vegetable powder or juice with up to 10,000 ppm nitrite, cherry powder as a source of ascorbic acid (a cure accelerator), and lemon powder which also accelerates curing through lowering the pH (Sindelar 2011). Cultured celery juice should be added such that there is approximately 50 ppm nitrite in the formulation to maintain antimicrobial effectiveness, antioxidant protection and to maintain cured color. Vinegar is buffered and adds additional natural acetic acid to the product. Sindelar (2011) reported that 2.0% buffered vinegar, a 3.0% cultured sugar/vinegar blend and a 1.5% lemon/cherry/vinegar blend each inhibited *Listeria monocytogenes growth* in uncured turkey and beef. In addition, these antimicrobials were as acceptable to consumers as conventionally cured frankfurters. Shrader (2010) demonstrated that *L. monocytogenes* was controlled in frankfurters when conventionally cured (156 ppm nitrite with 2.5% potassium lactate/diacetate), when naturally cured with vegetable juice powder (sources of nitrate or nitrite) were used with either 3.0% cultured sugar/vinegar or a 1.4% solution of cherry/lemon/vinegar.

12.10.1.2.1 Uncured Cooked Sausage

Uncured cooked sausage typically includes similar antimicrobials to those that are used in cured sausage. Since nitrite is not included, there may be antioxidants added such as BHA, BHT, and/or propyl gallate or natural antioxidants added through lemon powder, cherry powder or natural flavors. Many times, uncured cooked sausage is sold frozen as patties or links and therefore must have antioxidants that can prevent lipid oxidation that occurs during long-term frozen storage.

12.10.1.2.2 PSE Meat Use in Emulsion and Coarse-Ground Sausage Products

Genetic selection and pre-slaughter stress can cause rapid postmortem glycolysis that results in increased lactic acid production and decreased pH in pork and poultry meat. Decreased pH combined with high muscle temperature (Camou and Sebranek 1991) causes protein denaturation that exceeds that observed in normal muscle (Briskey and Wismer-Pederson 1961; Bowker et al. 2000) leading to the production of pale, soft, and exudative (PSE) meat.

The pale, soft, and exudative (PSE) condition was first identified in the pork industry in the 1950s and 1960s (Briskey and Wismer-Pederson 1961), and a similar condition was documented in poultry breast meat in the late 1970s (Froning et al. 1978). PSE meat has an undesirable pale color, soft texture, and surface moisture resulting in an unacceptable appearance and a dry, tough product after cooking. In addition, it is very difficult to utilize PSE meat in further processed products because the proteins (myosin and actin) that are responsible for the texture of deli meat and frankfurter type products are denatured (not functional), which leads to product failures such as cracking (deli meats) fatting out (frankfurter type products), and increased oxidation (O'Neill et al. 2003). Camou and Sebranek (1991) reported that slow heating of protein gels increased the functionality of PSE meat when compared to fast heating, but that normal pork had much higher gel strength than PSE meat. By slow heating of gels formulated with a combination of PSE and normal pork, it may be possible to increase functionality in emulsion-type products. In addition, Zhang and Barbut (2005) reported that tapioca starch increased gelation ability in emulsions made with PSE meat, and Kissel et al. (2009) reported that use of isolated soy protein, sodium tripolyphosphate, and cassava starch were effective at enhancing the functionality of PSE meat in the production of Mortadella. The most successful way to utilize PSE pork is through combining dilution with other techniques in sausage or deli products. The incorporation of binders and a percentage of PSE pork appear to be the most logical alternative for utilizing PSE pork or poultry in emulsified meat products. Utilizing 25% PSE meat and appropriate binders such as carrageenan, sodium caseinate, soy protein concentrate, or modified food starch can produce a product similar in quality to that with no PSE incorporated.

12.10.1.3 How to Reformulate for Reduced Sodium and Fat in Cooked Sausages

Reducing sodium and/or fat in product formulations leads to potential quality and yield challenges that are addressed through ingredient and processing technology. Sodium can be reduced by 25–50% in a cooked sausage product and labeled as "reduced sodium". Cooked sausage can only be labeled as "low" sodium if there is 140 mg or less sodium per 50 g of product (9 CFR 2011). This is not usually feasible with sausage since a threshold concentration of sodium chloride is needed for protein extraction and acceptable texture and flavor. However, fat-free, low-fat, and reduced fat cooked sausages can be manufactured through raw material selection and ingredient technology.

12.10.1.4 Formulating to Reduce Sodium in Cooked Sausages

Sodium can be reduced by 25–50% through substitution with potassium chloride, bitter blockers, and flavor enhancers (Desmond 2006; Schilling et al. 2008). For example, a frankfurter can be made by replacing 35% of the sodium chloride

concentration in the product with potassium chloride to obtain sufficient extraction of myofibrillar proteins. Sodium gluconate is included in the formulation as a flavor enhancer, an emulsion stabilizer, and to prevent the bitter taste that is often imparted by potassium ions. Sodium gluconate can also be used to decrease the amount of sodium phosphate that is used in the product. As in the majority of processed meat products, sodium lactate, and/or sodium diacetate should be used to extend shelf-life and inhibit *Listeria* growth. Potassium salt versions of phosphates and lactates can also be used to reduce sodium with no functionality issues, and may actually improve the solubility of these types of products. All other ingredients that would be included in the formulation are similar to those that are used to manufacture all frankfurter products. Other strategies for sodium reduction in cooked sausages include the use of hydrostatic pressure to improve protein functionality, flavor enhancers, pre-rigor meat, and/or a combination of these technologies.

12.10.1.5 Formulating to Reduce Fat in Cooked Sausages

12.10.1.5.1 Choice of Meat Raw Material

Reduced fat sausage products are formulated with meat tissue that is lean when compared to the raw material used in most sausages (Schilling and Smith 2012). For example, lean beef trimmings are used in reduced fat beef frankfurters, and skinless turkey thigh meat (or other red meat raw materials) is substituted for a percentage of mechanically deboned turkey in reduced fat turkey frankfurters. Many companies currently produce poultry sausages that are formulated from broiler or turkey thigh meat that are similar to their pork sausage products, but are lower in fat content. Coarse ground sausages are often reduced in fat through using a leaner raw material and similar spices with minimal changes to other ingredients. For example, companies that have traditionally made whole hog pork sausage now use chicken thigh meat that is a low-cost raw material to make lower-fat versions of the same products.

12.10.1.5.2 Ingredient Reformulation

In reduced fat sausage, there are predominantly two ingredients that need to be considered. More water is added to the formulation so that the sum of the added water and fat percentage do not exceed 40%. In addition, binders such as modified food starch can be added at 1.5–2.0% to bind the additional water in the formulation and replace fat. The starch that is used is modified to prevent water loss after heating and has a gelatinization temperature that is lower than the final internal temperature of the sausage. If the product is being reformulated to minimize fat content (such as fat-free applications), lean meat is used, the percent water is increased substantially over that of a reduced fat product, and modified food starch is included at a concentration that approaches the legal limit of 3.5% (9 CFR 319 2011). Other ingredients

that can be used in fat-free or low-fat sausage include broth and flavor enhancers such as autolyzed yeast or hydrolyzed vegetable protein. Other fat replacers can be used such as carrageenan, soy protein concentrate or isolate, whey protein concentrate, non-fat dry milk, and dietary fiber. All of these ingredients provide excellent functionality in reduced-fat cooked sausages through reducing shrink, binding water, and improving texture, but modified food starch is commonly used to minimize cost. Most other ingredients in the formulation will be similar between full-fat, reduced fat and fat-free sausages. Blending procedures may need to be adjusted to compensate for the improved protein extraction of the leaner meat block, while the incidence of fatting out may be reduced. There are two important concepts to remember in the production of reduced sodium and reduced fat products. First, salt and fat are essential ingredients in processed meat products since they impart functionality, texture, and/or flavor. Second, sensory acceptability and shelf-life testing must be performed on reduced sodium and reduced fat products since consumers are highly variable in what they deem acceptable and reducing sodium and/or fat content without maintaining quality will not lead to successful products in the marketplace.

12.10.1.5.3 Poultry Products, Chicken Nuggets and Chicken Patties

Pressed products are a type of restructured product that include chicken nuggets and breast patties and are generally made from whole muscle trimmings. In the US, nuggets and patties are commonly made from white meat and skin, but this is not the case in other countries where red meat is often preferred. The breast meat raw material is often extended with mechanically deboned poultry (MDPM) and dark meat to lower costs, but too much of this raw material (more than 30%) can lead to oxidative rancidity. Pressed products are high profit items but have a large initial cost since molds need to be purchased that the nuggets or patties can be pressed into. These products have large profit potential since the meat is extended with skin and breading, but can be more labor extensive since there are potentially more processing steps. These products also have the advantage of extended shelf-life by exposure to individual quick freezing (IQF) (Schilling et al. 2012). Finely comminuted (emulsified) poultry products include frankfurters, bologna, and loaf items. These products characteristically contain about 15% fat and are primarily manufactured from chilled or frozen mechanically deboned poultry. Mechanically deboned poultry is easily oxidized and must be chilled and used within 1 day of processing or frozen and used within 90 days. Nitrite, sodium phosphate, and sodium lactate are also generally added to the formulation to prevent oxidation in the final product. MDPM is the most economical raw material that can be used in processed poultry since during 2014, it sold for approximately $0.25/lb, which is much cheaper than other meat raw materials.

Either kappa or a mixture of kappa and iota carrageenan can be used in restructured products such as nuggets and patties. The carrageenan is often added as a dispersion in water (with a cation or carbohydrate carrier) after the protein has been

extracted from the meat. The product is then placed into a mold, breaded and heat processed. This type of product works well with 0.5% carrageenan and 2.0% starch, but carrageenan can also be used in combination with soy protein to make the product appear more like whole muscle. This type of restructured product can be extended with about 20–25% water, and xanthan gum or konjac flour can be added in a low-fat product to mimic fat properties. Use of kappa carrageenan in breaded products also imparts freeze-thaw stability since it helps prevent moisture from migrating from the meat into the breading.

Emulsified poultry products can be coated, cooked, and frozen. The emulsified batter is cooled to between −2.2 and −3.3 °C through use of ice or carbon dioxide snow. After cooling, the batter is placed into a hopper that pushes the batter into a mold plate and then onto a conveyer belt. The coating process may include predust, batter, and breading. Predust is an optional process in which a drum breader is used to place an initial layer on top of the emulsion to improve batter adhesion. Two types of batter are common. A tempura batter is used to coat the product, and an adhesion batter is used to adhere additional breading to the product. The batter is commonly applied in a submersion system where the formed nuggets or patties are submerged beneath the batter on a conveyer belt. After coating in an adhesion batter, breading is commonly used to coat the system. Multiple types of breadings exist including bread crumbs and flour breading. Breading can be applied through a recirculating breader that has a moving crumb bed to coat the bottom of the product and breading that falls from above to coat the top of the product. After coating, rollers are used to apply pressure so that the breading "embeds" into the batter. The product is then either par fried or fully cooked, usually in oil. If the product is fully cooked, it is better to use a two-step process so that the first step is at a higher temperature with a shorter time to set the coating and then a slightly lower temperature for variable time to fully cook the product (Owens 2001). The product is then frozen at below −40 °C and packaged. There has been an increased trend to bake these types of products, instead of frying, but it is more challenging to produce a high quality product.

12.10.1.5.4 Spreadable Sausage

Spreadable sausages include liver sausage and pate. These sausages are made from liver, meat or fat. Liver is the predominant emulsifier, but other emulsifiers are commonly added to stabilize the fat, including soy protein concentrates, skim milk powder, or sodium caseinate (Ugalde-Benitez 2012). In these products, approximately 50% of the meat block is from fresh liver. Formulations for liver sausage are reported in Pearson and Gillett (1996). One of these formulations consists of pork livers (50 lb), 30 lb of pork jowls, 15 lb of veal trimmings and 5 lb of bacon ends along with milk powder, salt, onion flakes, white pepper, msg, and sodium nitrite. When making liver sausage, it is very important to use fresh non-frozen liver since it is the predominant emulsifier. If fresh liver is not available, other lean protein will need to be added to help stabilize the fat. First, the livers need to be chopped and mixed with the spices,

salt, dry skim milk, and sodium nitrite. The mixing of the liver with the salt is very important so that the liver protein is extracted and able to stabilize and adhere to the fat. After mixing the livers and dry ingredients to a fine paste, the fatty meats (jowls, veal trimmings, and bacon ends) can be added to the chopper with the liver and ingredients and chopped to a paste. This is a challenging part of the process and some artisan expertise and trial and error is necessary to know how many times and how long to chop. Six to eight minutes is a good starting point. The next step is to either grind through the fine plate of an emulsifier or grind through a fine grinder plate. After this, the sausage is vacuum stuffed into moisture proof casings and cooked in a water tank at 160–170 °F for 1.5–2 h or until a final temperature of 150 °F is achieved.

12.10.1.5.5 Fish Sausage

Emulsified meat products can be made from fish products as well. Kin et al. (2013) used catfish nuggets and fillets to make frankfurters. Fish myofibrillar proteins are less stable to thermal treatments than myofibrillar proteins from terrestrial vertebrates. However, using fish proteins with calcium alginate can be used to produce stable emulsions (Sato et al. 2003; Ugalde-Benitez 2012). In the US market, a major challenge to the production of emulsified fish products are the high production costs since fish meats are more expensive than the meat tissue from terrestrial vertebrates that are commonly used in the production of emulsified sausage.

12.11 Summary

Though meat emulsions are not true emulsions, the property of emulsification is crucial to the stability of cooked sausage, bologna, chicken nuggets, liver sausage, surimi, and many other processed meat products. Grinding, chopping, and addition of salt is necessary to solubilize salt-soluble, myofibrillar proteins that serve as the predominant emulsifier in finely chopped, processed meat products. Without the inherent emulsifying capacity of myofibrillar proteins, it would not be possible to produce the majority of processed meat products. The non-polar portions of the protein orient themselves to the fat globules, and the polar portions of the protein orient themselves to the aqueous phase of the emulsion that contains water soluble proteins, water, and other meat proteins. Soy protein and sodium caseinate can be included in meat emulsions to bind water and meat protein and emulsify fat, but are limited in their use level in the United States and therefore only slightly contribute to emulsification. Other ingredients such as modified food starch and carrageenan are able to bind water and provide texture, which allows myofibrillar proteins to encapsulate fat and bind protein since the starch and carrageenan are able to tie up the majority of free water. The science behind emulsified meat products is well established, but there is currently a push for clean label meat products. For clean label emulsified meat products, salt-soluble proteins and salt will remain essential for the emulsification of fat.

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Chapter 13
Margarines and Spreads

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13.1 Introduction

The application margarine and spreads refers to a series of products, which are likened to butter, but have different fat contents. The definition of margarine is rigidly fixed with regards to fat content, a minimum of 80% by weight must be present, but the rheological characteristics of margarine can range from liquid to plastic in nature. Any edible oil or fat source may be used in its manufacture.

The definition of spreads is more ambiguous since they may contain a wide variety of fat contents, thus promoting the low fat, and reduced fat spread concept. This typically refers to anything between 25% and 70% fat content, but today modern demands often exist for even lower fat levels.

Margarine was invented in response to a request from the French Government of Napoleon III for a less expensive, longer life replacement for butter. The invention, credited to Hippolyte Mège-Mouriez, took place around 1860s and focussed on the rendering of tallow fat by artificial gastric juices, a crystallisation step at ambient temperature and extraction under pressure to obtain oleomargarine, a semi-fluid fraction and oleostearine, a hard white fat in the ratio of 60:40 respectively. The softer fraction was noted to have a flavour not dissimilar to butter fat, a similar melting point and a typical pale yellow colour, and the material could easily be plasticised. Thus, it represented a firm foundation material for the production of a butter substitute. The material was thought to contain glycerides of margaric acid, but it is now established that the fatty acid content is made up from palmitic and stearic acids—but nonetheless the name margarine has stuck.

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Using this fat source as the base, Mège-Mouriez mixed varying amounts of milk and water to the fat, stirred the mixture and formed a thick but stable emulsion, which upon further churning took on the consistency and resemblance of butter. Thus, the butter substitute that is margarine was formed, and essentially the production of margarine today follows the same basic trends.

As the patenting and production of margarine became established throughout the 1870s–1880s in both Europe and the US, not everyone was pleased by this new, 'anti-butter' arrival and opposition groups were formed to combat its use and application. These groups stemmed particularly from the farming and agriculture communities and ended up with anti-margarine legislation being adopted, which continued well into the twentieth century!

13.2 The Rise and Fall and Rise of Margarine: New Market Trends on Margarine Consumption Rates

The production of vegetable oils is high throughout the world, and consumption is increasing, especially among the lower socioeconomic groups. UN projected population growth is currently estimated to reach 9.7 billion by 2050, and suggests growth will be mainly in developing countries, with more than half in Africa (United Nations Department of Economic and Social Affairs Population Division 2015).

Numerous western countries have chosen to review health benefits of reduced SAFA foods (Wassell et al. 2010). Consequently, where possible, many food manufactures have reformulated products to deliver foods with lower calorie from SAFA, whilst attempting to maintain comparable textural qualities (Wassell 2013).

Butter, has always been the bench mark for margarine style products. In attempt to reduce both SAFA and *trans*, vegetable margarine type spreads may have experienced decline. Paradoxically, butter consumption (especially spreadable butter) has increased (Salter 2013). Global consumption of dairy products is expected to increase steadily in the coming years because of economic growth in emerging and developing countries (Asahi Shimbun 2015). In turn, in the regions of central, Southern Asia, central, South America, and many Africa nations, margarine consumption of both domestic retail and primarily industrial types is increasing. Why is this?

The fact is that there is a growing 'middle' income population in countries such as Indonesia, Vietnam, Myanmar, Thailand, Nigeria, India and China.

Consumption of cake, biscuit and snack foods is trending upwards in those countries. Margarine and shortening are in high demand. New state of the art manufacturing facilities are either built or coming on stream to cope with growing demand for these types of product. The same equipment is also being utilized to manufacture the insatiable demand for chocolate coating fats—otherwise known as cocoa butter substitutes (an alternative from Coconut oil or Palm kernel oil fractions).

While the demand for multipurpose margarines and shortening for bread, cake, biscuits, soups, sauces increases; equally, there is demand for more 'high end' products having improved melting behavior. The latter presents a logistics and distribution headache for countries situated within the tropics and equatorial region. To provide products, which either have lower melting points and or lower SAFA contents is a technological challenge when confronted with inadequate and inconsistent temperature controls during distribution or at point of use.

An additional factor to appreciate is that, there is a changing nutritional landscape, moving in alignment with UN guideline policy. E.g. As of 2015 all cooking oils sold within Indonesia must comply with a 'zero trans isomer' policy. It is anticipated this will eventually, not only be limited to cooking oil, but will also apply to margarines and shortenings.

So, the technical challenge will be to supply products free from *trans* fatty acids (Wassell and Young 2007), lower in SAFA and that are able to satisfy industrial food manufactures with adequate creaming performance and emulsification properties. Separately, the margarine and shortening (as a compound ingredient) should be able to withstand post-hardening—otherwise known as post-crystallization.

13.2.1 Supply and Demand

Currently, Malaysia and Indonesia have been using zero tax controls to maintain market position on exported palm oil, and its products (Mishra 2015). Meanwhile, the Philippines, currently a minor producer of palm oil, recently declared ambitions to convert some 8 million hectares of idle lands across the country into oil palm plantations (Reyes 2014). The reality is, as described earlier is that Palm oil production is set to grow even further.

Despite projections of slower economic growth in China and India (Indonesia's biggest palm oil importers)—export growth is expected to continue. Chinese and Indian palm oil import growth will continue due to inelastic demand fueled by population growth and increasing incomes. Despite slowing growth in GDP in China and India, Chinese GDP growth is expected to range between 8% and 11%, and Indian GDP will grow above 5% over the next 2 years (Thomson-Wright and Wiyono 2014).

13.2.2 Other Factors

Additionally, it must be understood that palm oil is the most suitable by volume; yielding edible oil crop per hectare. By majority, the availability of palm oil happens to be obtainable where population growth is set to be most prolific. Naturally, it may be assumed that palm plantation companies are or will be extremely interested to

capitalize on demand for downstream value products. Margarines and shortenings, will meet the growing demand for bakery products particularly in Asia. This may also open the possibility to supply liquid margarines or liquid shortenings for larger scale manufacture (Wassell 2006a, b, 2014). There are several reasons for this: (1) to minimize handling of packaging materials; (2) reduction of SAFA. In some cases these liquid systems could be used for direct replacement of a semi-solid margarine or shortening. In other cases, some reformulation of the product might be necessary. Either way, these liquid systems are standalone products, or are vehicle for other minor, functional ingredients, such as emulsifiers and enzymes.

It is interesting to note, that in the broad Indonesian domestic market, margarine is used not as a semi-solid product, but is usually used in the liquid form for both cake and bread manufacture.

13.3 Terms and Terminology

As with any branch of science the operating terms can be confusing to the layperson, and therefore the regulation of them is aimed to unify and standardise the field. The public tend to refer to spreads as margarine even when they are not, often in the ignorance that they are incorrect. The current description for the whole category is termed spreadable fats, and this includes butter. These spreadable fats are further described as a solid, but malleable emulsion, where the fat content must be at least 10% but less than 90%. This excludes the very low fat content of some of the water continuous spreads. Further physical specifications are placed on the spreadable fats in that they must be both solid and spreadable at a temperature of 20 °C, and more often they must be spreadable at refrigeration temperatures, i.e. <5 °C.

The definition of margarine has been established for over a century now, and is regarded as being similar to butter, i.e. it has a fat content of at least 80%. Anything under this fat content, by definition, is not margarine, but must be referred to as a spread, low fat or otherwise. Here, the modern legislation is complex and terms such as three-quarter fat, half-fat and fat content X% are routinely seen. Without entering into individual national semantics, it is fair to say that three-quarter fat refers to a fat percentage of 60–62%, half-fat to 39–41% specifically. Reduced fat falls within the range 41–62%, and low fat or light products under 39%. Overall, there is a general consensus that a fat spread product, be it butter, margarine or other must have a fat content between 10% and 90%. These options are summarised in tabular form in Table 13.1. It is worth stating that within the reduced to low fat region a range of ‘functional’ spreads are being routinely created to address cholesterol issues by adding sterol or stanol esters. Other trends see the incorporation of probiotic cultures for improving gut flora and general well-being, and there is the continued trend of increasing the content of specific functional fatty acids such as omega-3’s derived from marine sourced Long Chain Poly Unsaturated Fatty Acids (LCPUFA), and conjugated types.

Table 13.1 Product type versus their fat content in percent

Product type	Fat content %
Butter	80[a]
Margarine	80[a]
Three-quarter fat	60–62[a]
Half-fat	39–41[a]
Reduced fat	>41 to <62[a]
Low fat/light	<39[a]
Very low fat	20–30[b]
Water continuous	10–15[b]

[a]Article 5 of the Council Regulation (EC) No. 2991/94, laying down standards for spreadable fats
[b]Other industry classifications

13.4 Building Blocks and Structure

Margarine is classified as water-in-oil (W/O) emulsion. A W/O emulsion is characterised as having the water phase, the dispersed phase, being distributed within the fat or oil phase, the continuous phase, as droplets. We have established that the fat content of the margarine is equivalent to butter, 80%, but the moisture content is held to a maximum of 16% and the remaining 4% is a complex mix of proteins, emulsifiers, salts, flavours, colours and vitamins. Understanding the chemistry and mechanics of the fat phase is therefore important for producing a stable margarine/spread product.

The oil blends within margarines can be diverse, governed by market situations, price, availability and other factors. Hence, it is important to be able to utilise different oils as circumstances dictate, and therefore it is necessary to understand the physical properties of the oils and fats being used, i.e. their crystallisation rates, melting properties as well as solid/liquid fat ratio.

13.4.1 Oils and Fats

Oils and fats are triacylglycerols (TAGs), and are liquid and solid at ambient temperatures, respectively. This is shown in example form in Fig. 13.1 for Monoacylglycerols. The prefix, *sn* denotes stereo-isomerism, comprising glycerides with glycerol shown with the secondary hydroxyl group on the left and the carbon numbered 1, 2, and 3 from the top.

A triacylglyceride (TAG) consists of glycerol esterified with three fatty acids, which can either be three similar ones, called a simple TAG, or two or three different ones, in which case it is a mixed TAG. A schematic example is given in Fig. 13.2 (Madsen 2003).

As can be seen in Fig. 13.2, double bonds are present in some of these fatty acids. Modification of the fatty acids, usually by means of hydrogenation, is where the unsaturated fatty acids are transformed into saturated fatty acids. Here, an example could be $C_{18:1}$ (oleic acid) going to C_{18} (stearic acid). Such modifications offer the oils and fats manufacturer a greater flexibility and the chance to dramatically alter the melting point of the fat. The fatty acid composition of some natural fats along with other important information is summarised in Table 13.2. Given current trends, the down side to hydrogenation is that during the addition of hydrogen, *trans* fatty acids, which are schematically shown in Fig. 13.3, are formed. Selective hydrogenation involves the saturation of the most polyunsaturated fats first, such that the *trans* fatty acid concentration increases up to a point until they themselves are hydrogenated. If the reaction runs to completion, then the *trans* isomer is absent. The *trans* fatty acids can have substantially higher melting points than the corresponding *cis* fatty acid, where the difference can be in excess of 30 °C.

Over and above the physical aspects of *trans* fatty acids, ruling in the United States, valid from 1st January 2006 required all food stuffs to have the *trans* fatty acid content labelled. This requirement is in response to studies that show human

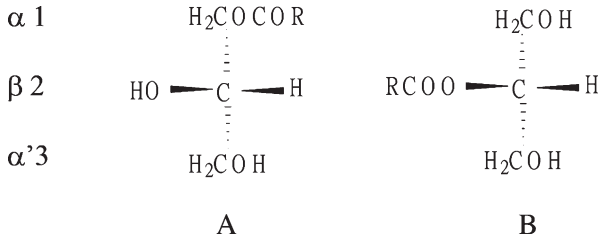

Fig. 13.1 Monoacylglycerols, where A is 1-monoacyl-*sn*-glycerol (α isomer), and B is 2-monoacyl-*sn*-glycerol (β isomer)

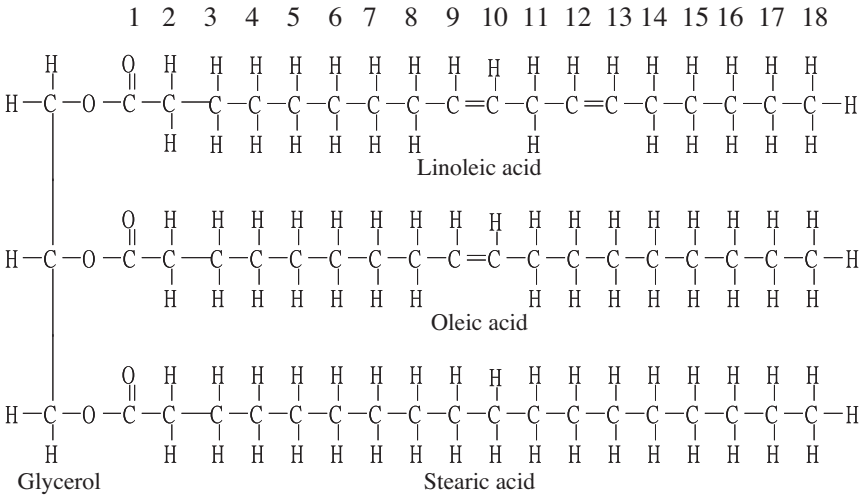

Fig. 13.2 2-Oleolinoleostearin

Table 13.2 Composition of fatty acids in vegetable oils and fats, together with other important information

Fatty acid	Castor oil	Coconut oil	Palm kernel oil	Palm oil	Ground-nut oil	Cotton-seed oil	Soya bean oil	Sunflower oil	Linseed oil	Rape-seed oil
Caproic C_6		0.5[a] 0.2–0.8[b]	0.5 T–1.6							
Caprylic C_8		8 6–9	4 3–10							
Capric C_{10}		7 6–10	5 3–14							
Lauric C_{12}		48 44–51	50 37–52							
Myristic C_{14}		17 13–18	15 7–17	2 0.5–5		1 0.5–2	T	T		0.5 0–1.5
Palmitic C_{16}	2 1–2	9 8–10	7 2–9	42 32–47	10 7–12	21 20–27	8 7–10	6 4–8	5 4–7	2 1–4
Stearic C_{18}	1 1–2	2 1–3	2 1–3	5 2–8	3 2–6	2 1–3	4 3–6	4 2–5	4 2–5	1 0.5–2
Arachidic C_{20}			T T–0.6		3 2–4	0.5 0.2–1	0.5 0–2	0.5 0–1	T	0.5 0–1
Behenic C_{22}					2 T–3			0.5 0–1		1 0.5–1.5
Lignoceric C_{24}				T	2 1–3				T	1 1–2
Palmitoleic $C_{16:1}$		0.2 T–0.4	0.5 T–0.6		T	0.5 0–2	0.5 T–1	T		T
Oleic $C_{18:1}$	7 T–8.5	7 5.5–7.5	15 11–23	41 40–52	50 35–70	29 22–35	28 20–35	28 20–40	22 12–34	15 11–24
Gadoleic $C_{20:1}$										7 5–12

(continued)

Table 13.2 (continued)

Fatty acid	Castor oil	Coconut oil	Palm kernel oil	Palm oil	Ground-nut oil	Cotton-seed oil	Soya bean oil	Sunflower oil	Linseed oil	Rape-seed oil
Eurcic $C_{22:1}$										50 40–55
Ricinoleic $C_{18:1}$	87 86–92									
Linoleic $C_{18:2}$	3 3–6	1.3 T–2.5	1 1–3	10 5–11	30 20–25	45 42–54	53 40–57	61 45–68	17 14–20	15 11–29
Linolenic $C_{18:3}$						1 T–2	6 5–14	T	52 35–65	7 6–13
Pure glycerine	8.8–9.8	13.2–13.5	12.2–12.8	5.5–10.0	8.7–9.9	10.6	10.2	10–12	0.4–10.5	9–9.7
I_2 value (Wijs)	81–91	7.5–10.5	14–23	44–54	84–100	99–113	120–141	126–136	55–205	97–108
Saponification value	177–187	250–264	245–255	195–205	188–195	189–198	189–195	186–194	188–196	170–180
Melting point (°C)	–10 b. –12	23–26	24–26	27–50	–2	–2 b. +2	–20 b. –23	–10	–20	–9
Titer (°C)		20–24	20–24	40–47	26–32	30–37	20–21	16–20	19–21	11–15

T trace (Madsen 2003)

[a]Refers to the typical value

[b]Refers to the range due to natural variability—valid throughout the entire table

intake of *trans* fatty acids, similar to that of saturated fatty acids increases the concentration of low density lipoprotein cholesterol (LDL-C) in the blood. This is colloquially referred to as the 'bad cholesterol'. The regulation states that the content of the *trans* fatty acids must be recorded to the nearest gram if the serving contains 5 g or more of the fatty acid. If the content is below 5 g then the *trans* content must be declared to the nearest 0.5 g and if the content is below 0.5 g then it can be declared 0 g, and "not a significant source of *trans* fat" may be used. Herein, there is still scope for margarine technology to play a part in delivering less fat per serving and actually allows the use of higher *trans* content oils (Klemann 2004)!

Table 13.2 gives the melting points of the individual oils that are used to make up the fat blends, but it is also important to know and recognise the melting points of the fatty acids themselves. These are outlined together with the number of double bonds they contain in Table 13.3.

In tackling the issue of reducing *trans* saturates, palm oil offers a natural solution and has grown widely in use and status since the 1990s (Wassell and Young 2007). Unlike the majority of vegetable oils, e.g. soybean, rapeseed, sunflower oils; palm oil does not necessarily require a hydrogenation step, which can lead to formation of (dependant on degree of hydrogenation) *trans* isomers (Wassell and Young 2007). The whole palm oil, already, naturally contains significant amounts of saturates (Wassell et al. 2010). While palm oil is not necessarily regarded as a low saturated fat, it can easily be fractionated, resulting in more unsaturation, e.g. olein fractions. Palm is probably the most important oil in terms of fractionation because of its unique fat profile (Sahasranamam 2005) which can be broken down into many individual fractions which are naturally more or less saturated (Timms 2007).

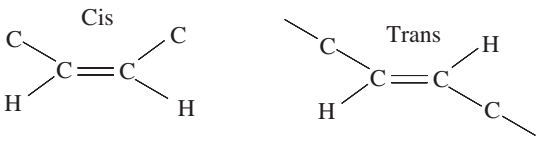

Fig. 13.3 Schematic diagrams of *cis* and *trans* configurations

Table 13.3 Common fatty acids showing their melting points and number of naturally occurring double bonds

Fatty acid	No. of double bonds	Melting point (°C)
Lauric C_{12}	–	44.2
Myristic C_{14}	–	54.3
Myristoleic $C_{14:1}$	1	Liquid
Palmitic C_{16}	–	62.9
Palmitoleic $C_{16:1}$	1	Liquid
Stearic C_{18}	–	69.6
Oleic $C_{18:1}$	1	16.2
Linoleic $C_{18:2}$	2	Liquid
Linolenic $C_{18:3}$	3	Liquid
Arachidic C_{20}	–	74.4

13.4.2 Palmitic Acid as a Component of Total SAFA Consumption

Recently, there has been much debate as to the quantity of total saturated fatty acids (SFA) in the human diet (Benatar and Stewart 2014; Schwingshackl and Hoffmann 2014). New possibilities to design and structure triacylglycerol (TAG) based systems have been hypothesized (Wassell 2013). More recently, the debate has drawn attention to the specific type of SFA in the TAG structure (Chowdhury et al. 2014).

One opinion, is that SFA of the C_{12}, C_{14} and C_{16} are the main culprits to cardiovascular disease (Legrand 2013); recommending to distinguish among the saturated fatty acids, the subgroup of 'lauric, myristic and palmitic acids' [because these are] considered to be atherogenic in case of excess consumption. Legrand (2013) presents the argument that C_{16} is 'worse' (higher), once hydrogenated. To avoid misunderstanding, there needs to be accurate context. A higher concentration of C_{16} can be achieved by the fractionation process, as shown in Table 13.4, avoiding the hydrogenation process completely. However, the real argument is that while the level of C_{16} may be higher or lower, depending on the particular food system, the actual inclusion levels will vary from application to application. For example, a very saturated palm stearin product characterised in Table 13.4, would only be used at 'minor' concentrations in the final food product. The physical behaviour and chemistry of a selected fat is such as to enhance structure and crystallisation. Their inclusion is to provide a preferred sensory quality, usually associated to the food item, e.g a proportion of highly saturated fat could be added to a ratio of unsaturated oils to achieve desirable melting (sensory) characteristics usually, targeted at, e.g. 1–5% max at 37.5 °C. These fats would not normally be consumed in their pure form. Additionally, these could be added into the said food product to aid, e.g Pickering stabilisation of an emulsion. The final effect helping to achieve a desired eating quality, with a specific rheological characteristic.

Palmitic (C_{16}), is the most abundant in nature and a major SFA of the Western diet (Hornstra 2014). Within the EU palm oil is a major source of C_{16} followed by dairy products. The whole diary butter fat contains approx. 31% Palmitic (29–34%). Separately, and important to mention—cocoa butter fat contains approx. 27% pal-

Table 13.4 A comparison of the typical specification of fully hydrogenated Palm Stearin and dry fractionated Palm Stearin

	Fully hydrogenated palm stearin	Dry fractionated palm super stearin
Iodine Value (IV)	2 max	10–15
Fatty acid composition		
C12:0	1.0 max	1.0 max
C14:0	3.0 max	3.0 max
C16:0	48–63	75–85
C18:0	32–37	4–8
C18:1	Trace	8–10

mitic (24–30%). Both functionally and nutritionally, the specific position of the fatty acid (namely palmitic acid) in the TAG structure is fundamental.

Siri-Tarino et al. (2010), suggest SFA consumption per se is not associated with increased cardiovascular risk. Recently, a study on the *trans* and saturated fats levels and its effects on plasma fatty acids by Benatar and Stewart (2014), suggested that a "Change in plasma levels of fatty acid isomers did not correlate with the amount of that fatty acid in dairy food. For example, myristic acid and palmitic acid were the most plentiful saturated fats in milk. Changing dairy food intake did not correlate with change in plasma levels." This research found no significant change in total saturated fats for increased dairy diet +13.1 (95% confidence interval (CI) –1.2 to + 25.3)%, same dairy diet +2.4 (95% CI –9.2 to +11.3)% or decreased dairy diet +3.5 (95% CI –5.5 to +11.6)%.

Cambridge researchers (Chowdhury et al. 2014) who re-analysed 72 separate studies (covering 600,000 participants from 18 countries), studied the effect of total saturated fatty acids in two ways: first as the component in the participants diet and second within the bloodstream. Collective analysis revealed that whether measured in the diet or in the bloodstream, total saturated fatty acids were not linked to coronary disease risk. When looked in detail, they found a weak link between bloodstream levels of palmitic and stearic acids and heart disease.

In respect to Type 2 diabetes, researchers suggest even-chain saturated fats (such as myristic, palmitic, and stearic acids) originate endogenously from lipogenesis from carbohydrates and alcohol in liver or adipose tissue (de Souza et al. 2015). Another study showed much of the saturated fat in the diet was provided by palm oil and milk fat rather than meat fats (Sanders et al. 2013). The study concluded no clear benefit when replacing SFAs with either MUFAs or carbohydrates.

Based on current research, de Souza et al. (2015) have shown saturated fats are not associated with all-cause mortality, CVD, CHD, ischemic stroke, or type 2 diabetes; but do clearly show *trans* fats are associated with all-cause mortality, total CHD, and CHD mortality, probably because of higher levels of intake of industrial *trans* fats than ruminant *trans* fats. The same authors recommend dietary guidelines should carefully consider the health effects of recommendations for alternative macronutrients to replace *trans* fats and saturated fats.

13.4.3 Fat Crystallisation

This is a hugely important area, which basically governs the texture of the margarine or spread. Topics discussed in this section will cover aspects of crystal form, crystal size and crystal binding. Understanding the crystallisation procedure will ease the processing of the individual oil or fat since there can be differences and variations in crystallisation rates from batch to batch and there are differences from oil to oil.

Fat crystals are polymorphic, having the following forms, α, β′, and β, where the melting point increases in the respective order written. The difference in melting

points between the different fatty acids can be large, such that for $C_{18:3}$ (Linolenic acid) to C_{22} (Behenic acid) the difference in melting point of the β form is more than 100 °C. The conformation of the fat crystals can be viewed as being like a chair (van Soest et al. 1990) and they are packed in units of two. These are schematically shown in Fig. 13.4, where Fig. 13.4a shows the structure of fats normally used in the margarine industry whereas Fig. 13.4b shows that for cocoa butter. Also present in Fig. 13.4 is an indication of the long spacing (LS), which is the length of the TAG unit in the TAG row of a crystal. The angle of tilt, t, will depend on the LS value such that larger LS values result in smaller angles of tilt and vice versa.

The short spacings, shown schematically in Fig. 13.5, represent distance between the fatty acid chains, and these can accurately be measured by X-ray crystallography. The typical values of the short spacings of the three crystal types are: α—4.15 Å, β′—3.8 and 4.2 Å, and β—4.6 Å. α, β′, and β crystals (Hoerr 1960) can be formed directly from the melt, or α to β′ to β, but this is not reversible. By measuring the short spacings between the fatty acids, one can ascertain and quantify the type and

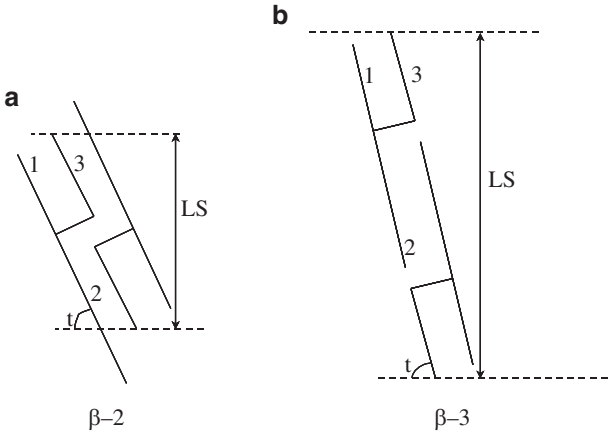

Fig. 13.4 Arrangement of TAG molecules in the (**a**) β-2, and (**b**) β-3 modifications, where LS is the long spacing, t is the angle of tilt, and 1, 2, and 3 represent the TAG configuration

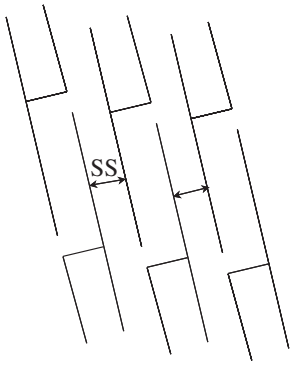

Fig. 13.5 Fatty acid units showing the short spacing between the individual fat units

ratio of the fat crystal forms in a given blend. Through similar techniques it has been established that margarines and spreads are preferred with crystal polymorph that exists in the β′ form. The influence of processing however can have dramatic impact on crystallisation kinetics. For example, if the fat blend contains beef tallow, then the crystals are β′ in margarine made with a tube chiller, but β in margarine made with a chilling drum. Palm oil is probably the most widely used of vegetable oils, and it is naturally β′ tending largely because of its diverse fatty acid profile, and particularly high content of palmitic acid (Berger and Idris 2005). However, if processed incorrectly, these benefits are lost, and because palm oil also contains unusually high content of diglycerides ~6–7%, the diglycerides negatively influence crystal kinetics (Siew 2002). Therefore the correct processing parameters must be found when using palm oil.

The different crystal forms, α, β′, and β each have their own physical configurations (Hernqvist 1988), and it is well known that fat crystals with similar chain lengths, e.g. hydrogenated sunflower oils transform more quickly from the β′ to β form. This is similarly true for hydrogenated low euric acid rape-seed oil (Yap et al. 1989). This property is attributed to the uniform end layers between the triacylglyceride rows in the crystal. It is similarly well known that Sorbitan tristearate (STS) esters co-crystallise with the TAG, and because of their irregular molecular form compared with TAG, prevent the 90° rotation of the TAG, thus helping to delay transformation from the β′ to β form (Madsen and Als 1968).

As the crystal form changes, the texture likewise changes. This typically takes place under storage and the usual transition is from β′ to β. During this transition crystal size increases dramatically, from ~3–5 to ~100 μm respectively, as does melting point. The result is that the margarine now has a sandy/gritty type mouthfeel (Kristott 1999).

The crystals in margarine, spreads and shortenings are bound together by a network of crystal-to-crystal contact bindings. The functionality of the semi-solid margarine, termed plastic fat, is influenced by the ratio of liquids to solids in the lipid phase, and the crystal packing arrangement developed during processing (Timms 1991). Control of crystal form, size, and shape must be balanced with careful blend selection, and are critical for final application in bakery products. Often these inter-crystal associations are classified as primary (irreversible) and secondary (reversible) bindings, which can be reliably measured using creep—recovery techniques (Marangoni 2005).

A high content of secondary bindings is desirable in puff pastry type margarine because they allow the margarine to maintain a high degree of plasticity under rolling of the puff pastry dough. A degree of both primary and secondary bindings is beneficial in cake margarine so that the margarine becomes soft, thus facilitating air incorporation during whipping with sugar.

13.4.4 Oil Modification: Methods and Implications

Probably all oils and fats will undergo some form of modification before use in a food product. The most well used modifications are hydrogenation, interesterification and fractionation. Depending on the naturally occurring starting point of saturation or unsaturation of the said oil, the degree of modification might result in a more saturated or unsaturated fat having a higher or lower melting point than the starting material. The importance of this cannot be overemphasised, because it provides structure, texture and hardness to the food product, lubrication, and in terms of emulsion stability, can provide specific crystallisation behaviour, necessary for continuous fat phase dispersions/emulsions. The extent and degree of satisfying these criteria are dependent on the amount of solid fat, size, shape and interactions of the crystals (Wassell and Young 2007). Figure 13.6 shows schematically, the degree of modification, liquid oil is required to obtain the desired solid fat structure, which could be used, for example, in margarine or shortening production. Detailed reviews about the subject of structuring edible fat based systems are discussed by Wassell and Young (2007) and Wassell et al. (2010).

The hardness or consistency is a measurement of the texture of the margarine. It may be linked to the type of fats and oils used and the process conditions. There are strong correlations between the SFC% and the plasticity of margarine. This also has implications for the overall melting characteristics of margarines and spreads at various temperatures.

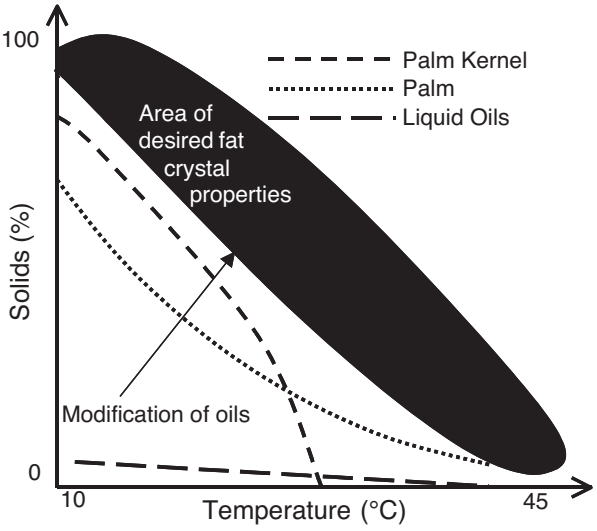

Fig. 13.6 Desired solid phase lines for hardstocks (dark area) compared to naturally occurring oils and fats. (Adapted from van Duijn et al. 2006)

The determination of a required SFC profile, must be factored according to the local climatic, distribution chain and performance demanded in the margarine's application (Yusoff et al. 1998).

Ideally, the SFC, melting profile, and crystallisation process is such that texturally the fat blend gives the optimal structure and sensory properties characteristic of table margarine or spread (Chrysan 2005).

13.4.5 Choice of TAG as Raw Material

Palm oil is the most abundant natural edible vegetable oil in the world and yet, most consumers probably do not intuitively associate palm oil as being "natural". In light of an increasingly health-conscious generation, consumers need to make informed choices. Therefore the question is: why is palm oil important for health and nutrition? In short, the oil of Palm has a unique composition making it *trans* fat free. Many health authorities, including the World Health Organisation (WHO), now advise against *trans* fats because it is clearly proven to increase cardiovascular disease. In fact, the US Food and Drug Administration has recently ordered industrial *trans* fats to be removed from all food supply by 18 June 2018; WHO also plans to eliminate it from food completely by 2023 (WHO 2019).

Palm oil, has become the most versatile alternative to other vegetable oils because in its naturally-occurring form, it is already semi-solid at room temperature, eliminating the need for partial hydrogenation, making it naturally *trans*-fat-free. This is one reason why, Palm oil has contributed significantly to nutrition in food products. Three decades ago, recognising the negative health impact of *trans* fat, a handful of global nutrition-focused food manufacturers actively sought to remove industrial *trans* fatty acids from their brands. For example, in the early 1990s, Unilever decided to remove *trans* fatty acids from spreads and other foods (Korver and Katan 2006). Efforts to use palm oil, played a fundamental role in ensuring a successful transition to producing food without *trans* fat. Since the middle of the 2000s, many countries started following WHO's guidance to mitigate *trans* fatty acids. The most natural and effective route is still palm oil. Concomitantly, came the genetic modification of soybean oil, triggering an increase of demand for more natural oils. Palm oil was the only natural edible vegetable oil in abundant supply. This unique status of palm oil remains today—currently, no other globally available edible oil rivals natural palm oil in its versatility, without need for modification, be it genetic modification, or modification through hydrogenation. Putting this into context—providing sustainable food in the Anthropocene is never more urgent. Many environmental systems and processes are pressed because food production is not operating efficiently. Providing healthy sustainable food systems will require large reductions in food loss and waste, together with improved food production, and agricultural practices (Willett et al. 2019).

13.5 Emulsifiers

Emulsifiers are necessary in all types of margarine and spreads to stabilise the liquid emulsion by reducing the interfacial tension between the fat and the water phases. The emulsifiers secure a fine and stable water phase dispersion, thus ensuring a homogeneous margarine/spread product with good functional, and overall microbiological keeping properties (Bot et al. 2003). Different types of margarine require different emulsifiers depending on which criteria are to be met. In frying margarine, water droplet size and distribution are controlled to thereby minimise spattering, which tends to plague this application (Chrysan 1996). In reduced fat spreads, the water content is higher than in retail table margarines and therefore the emulsifier is used primarily to bind the water and secure a stable reduced fat spread. For all-purpose, full-fat table margarine, and industrial cake margarine, a requirement of the application is that the emulsifier should impart good whipping properties. Therefore, it is essential for the emulsifier to ensure a good volume and uniform structure within the cake dough mix (Tamstorf et al. 1986). The cake recipe and flour type, and method of manufacture may similarly influence the type of emulsifier chosen for the margarine.

Common to all though, is the dispersion of one phase as large droplets within the other phase. In this case we refer to the W/O dispersion, as mentioned earlier.

The stability of the W/O emulsions is kinetic as opposed to thermodynamic, i.e. the system is thermodynamically unstable. If the system was to be thermodynamically stable, the emulsion should spontaneously reform after mechanical separation by means of centrifugation. However, experience shows that systems separated by centrifugation tend to remain that way unless mixed by external forces. In truth when the emulsion is separated into its two distinct phases, this is its naturally most stable state, and indeed the state towards which it will tend, over time. Hence, a stable emulsion is almost a contradiction of terms and basically refers to a system where the inevitable phase separation has been severely retarded such that it is imperceptible over the shelf life of the product, even if this is a period of years!

A range of emulsifiers are available for use in margarine and spread systems, and as the fat content is reduced and enters the low fat spread area, stabilisers for the water phase will also be required. Taking the emulsifiers first, one can choose from distilled mono, di, TAG, polyglycerol esters, lactic acid esters, citric acid esters, polyglycerol-polyricinolineate, propylene glycol monostearate, and Sorbitan tristearate, among others.

The distilled monoglycerides are sourced from refined and commercially available edible oils, such as sunflower oil, palm oil, rape-seed oil, vegetable oil, soy oil and animal sources, and work generally as an all-purpose emulsifier. They stabilise the liquid emulsion in water-containing systems by reducing the interfacial tension between the fat and the water. Simultaneously, they prevent syneresis in aerated and hydrated systems as well as facilitating the incorporation of other ingredients into the fat fraction. Coalescence can also be minimised, as in the case of frying marga-

rines. Depending on the selected emulsifier, it can also improve textural properties, resulting in a less waxy mouthfeel.

The lactic acid esters of mono-diglycerides are produced by reacting a full or partially hydrogenated vegetable oil with lactic acid to one or several hydroxyl groups. Its function is more in the final application than necessarily in the margarine fraction itself. Incorporation of the lactic acid esters reduces the whipping time required for creams, and cake batters, and it increases the degree of overrun obtained and improved overall foam stiffness. Lactic acid esters will also improve crumb firmness in the baked cake over time.

The citric acid esters of mono-diglycerides are produced from edible refined vegetable fats or sunflower oil, and are primarily used as replacers for lecithin. Through the citric acid esters the fat fraction and solids fraction becomes efficiently integrated resulting in a smooth, homogeneous and easy to handle system. Within margarine products themselves the citric acid esters are excellent anti-spatter agents.

By treating individual applications special focus can be placed on the type of fat blend used, the conditions that are required from the margarine or spread and therefore the emulsifiers that are chosen to meet these requirements. Independently, there are a wide ranging processes and dynamic conditions that also require discussion; this has already been adequately dealt with by Flack (1997).

13.6 Industrial Cake and Cream Margarine

This margarine is used in pound cakes, fillings, and short crust pastry, etc., and by nature of the products need to have air incorporated into them. This requires the margarine to work at the temperatures of usage and also allow the incorporated air to be retained within the structure of the cake batter. Similarly, the margarine should prevent the formation of long chains of gluten networks, thus ensuring the final product is crumbly to the bite.

Here the fat blend must provide stability over a wide temperature range, but must ensure the margarine is soft and easy to work with, and easily disperses into the cake batter, whilst imparting optimum stability to the cake batter. The stabilising effect takes over during baking, whereby the unit structure of the final cake becomes fixed because of gelatinised starch being cemented together with the protein matrix. The stabilising effect of the fat during batter preparation now serves as a lubricant mechanism, coating the individual flour particles, thus preventing them from forming extended gluten network formations.

Firmer cake margarines are available for cookies and biscuits, where the aeration capability may or may not be important. Ease of the margarine's incorporation into the batter is still of primary importance.

Lauric oils, i.e. those from coconut oil and palm kernel oil, are well known to have good whipping properties because they are by definition, high in short chain $C_{12:0}$ fatty acids. However, lauric oils are also known to be prone to hydrolytic rancidity, i.e. lipid oxidation (Britannia Food Ingredients Ltd. 2000), which imparts an

unpleasant flavour to the margarine but also the final product. This can be solved with antioxidative ingredients, which will be dealt with later in a separate section. Modern refining techniques are able to overcome this problem.

The overall fat content of cake margarines has tended to decrease in recent years, with the advent of low fat products, down to 60% fat products. However, reducing the fat content increases the water content and thereby reduces the whipping ability and baking properties of the final cake. Hence, additions of other ingredients to bind the water and essentially act as fat substitutes are required. The ingredients of choice are hydrocolloids, which stabilise the water phase, and allow for cakes to be made with similar volume and crumb structure to the standard 80% fat versions.

In order to achieve the above, usually it is necessary to use more than one emulsifier and typically a combination is used to achieve the optimum performance from the margarine. These combinations are readily altered as the conditions of the margarine performance changes. Typical combinations are given in Table 13.5.

When cake margarine is whipped together with powdered/granular sugar the recommended emulsifier blend is a combination of distilled monoglycerides, fully hardened, with either polyglygerol esters or lactic acid esters of mono-diglycerides. This combination ensures excellent cream volume within the cake mix. However, when the margarine is whipped together with syrup sugar or sugar with water to form creams, then unsaturated monoglycerides are recommended to maintain the desired structure of the cream. Typically, a relatively high IV (90–100) provides better performance because it affects both fluidity and emulsion/dispersion stability (O'Brien 1998). Creams produced with low IV mono- and diglycerides produce tight emulsions, and restrict aeration (Wassell 2005). Low IV mono- and diglycerides are more suited for cake formulas, and are normally assisted by a co-surfactant to aid other positive effects on final cake quality, as stated previously.

Table 13.5 The type of emulsifier combinations for cake margarine types under different conditions together with approximate dosage guides

Application	Emulsifier combination	Dosage
Cake margarine	Polyglycerol ester + fully saturated distilled monoglyceride	0.5–1.0% 0.2–0.5%
	Lactic acid ester + fully saturated distilled monoglyceride	0.5–1.0% 0.2–0.5%
	Propylene glycol ester + fully saturated distilled monoglyceride	0.5–1.0% 0.2–0.5%
Cream margarine whipped with granulated sugar	Fully saturated distilled monoglyceride + polyglycerol ester	0.1–0.2% 0.5–1.0%
	Fully saturated distilled monoglyceride + lactic acid esters	0.1–0.2% 0.5–1.0%
	Fully saturated distilled monoglyceride + polyglycerol ester	0.1–0.2% 0.2%
Cake margarine with syrup sugar	Unsaturated distilled monoglyceride	0.5–1.0%

13.7 Puff Pastry Margarine

The requirements for puff pastry margarine are quite different from the cake margarines above. Production of puff pastry involves the basic dough being rolled out and partly covered by a single, thin, flat piece of margarine. The uncovered dough is then folded over the margarine and the whole piece rolled out thinly. This folding and rolling procedure is repeated a number of times until the desired number of laminar layers of alternate dough/margarine is achieved. The whole process is known as lamination. By means of the Scotch method, the margarine is broken or cut into lumps and mixed together in the basic dough. The French method uses a whole piece/block of puff margarine, and this is enveloped into the dough piece unit, then laminated. The English method is where slices of puff margarine are placed over two-thirds of the rolled out rectangular dough piece, and then folded in a fashion which keeps dough/fat layers separate, and then laminated as previously described. Here the main function of the margarine is to separate the layers of dough and produce a pastry with a uniform flaky texture and a high volume. As each layer of margarine must be homogeneous and unbroken, it is extremely important that the margarine can withstand vigorous stretching and rolling, i.e. the margarine structure must be highly plastic. The fat blend used for the margarine must impart the necessary plasticity and typically involves the use of palm oil, tallow, and rearranged lard, where the solid and liquid fat content are balanced to give the plasticity desired over a wide temperature range.

The emulsifiers that are used to stabilise the puff pastry margarine act as all emulsifiers do, stabilising the liquid emulsion by reducing the interfacial tension between the water and the fat phases. However, the emulsifiers also play a role in the crystallisation of the fat during cooling, kneading, and storage processes. All this is optimised towards giving the margarine the required level of plasticity. Enormous processing pressures are typical for puff pastry manufacture, sometimes up to 100 bar pressure. Emulsifiers help to ensure plasticity by helping to secure and maintain water droplets, and they improve the heat stability of the emulsion during the baking process. The emulsifiers recommended for puff pastry margarine are given in Table 13.6.

As well as the emulsifiers recommended in Table 13.6 addition of lecithin at a dosage of 0.5–0.8% will help to extend plasticity.

Table 13.6 Recommended emulsifier blends and dosages for puff pastry margarine

Emulsifier blend	Dosage
Monodiglyceride/polyglycerol ester blend	~1.0%
Fully saturated distilled monoglyceride	~1.0%

13.8 Industrial Fillings

Fillings, in this context, refer to fat-based fillings such as those found sandwiched between biscuits, cakes, snack bars or the classic Swiss roll. The fillings are either added to an already baked product by injection, or are simply spread on the surface. A good filling must be easy to handle, stable—often at room temperature, and possess the fine plastic texture under storage but also melt quickly in the mouth. The fat blend must therefore reflect these demands, with a careful balance between the solid and liquid fat fractions. It must crystallise shortly after depositing, allowing another biscuit say, to be placed on top without the filling squeezing out the side. Current trends show that fat-based fillings of this nature fall into three main categories, standard fat fillings 20–40% fat; aerated filling stable at ambient temperature, 20–40% fat; and milk-based aerated filling, 20–35% fat. Each category has specific emulsifier demands.

The standard fat fillings of 20–40% fat content are the simplest and consist basically of fat and sugar. Their texture can be improved by addition of an emulsifier. Here the use of an unsaturated distilled mono-glyceride is recommended, as previously explained in the cake and cream margarine section. A smoother, more homogeneous filling is achieved that in turn incorporates and retains air.

For the fully aerated filling, which should be stable at ambient temperature with a fat content of 20–40%, other emulsifiers are required. Here, a combination of lactic acid esters of mono- and diglycerides together with citric acid esters of mono- and diglycerides is recommended. The lactic acid esters ease the incorporation and retention of air into the low-fat filling, simultaneously improving stability and stiffness. Reduction in whipping time required is also observed. The citric acid esters enable the integration of the fat phase with the solid/sugar fraction, and serve to give a smooth, easy to handle, homogeneous filling.

Milk-based aerated fillings of 20–35% fat content similarly have air incorporated into them, and can be characterised by their light, fluffy mouthfeel. Due to their higher water content, they are usually stored at refrigerated temperatures and the emulsifier used to obtain a stable emulsion and prevent water separation is an unsaturated distilled mono- and diglyceride together with a lactic acid ester based emulsifier, although this is not enough on its own. The water phase is further stabilised by hydrocolloids, which increase the viscosity and/or bulk to the water phase in addition to imparting stability and firmness to the final filling.

13.9 Reduced–Low Fat Spreads

As indicated above, reduced-fat and low-fat spreads typically have fat contents of 60% and 40%, respectively. The reduced-fat systems have to some extent been covered in the previous application areas, but the low-fat spread systems are used almost primarily for spreading on bread.

As the fat content is much less than in the systems already discussed, the demands on the emulsifier are greater, such that they must play an increasing role in the stabilisation of the water phase. The preconditions for a stable low-fat spread are small water droplets and a stable emulsion. Other components in the system, such as milk proteins, act to give a more open emulsion resulting in improved flavour release; but they also make controlling the water dispersion more difficult, with the consequence of shorter shelf life. The recommendation for which emulsifier to use therefore depends not only on the fat content of the spread product, but also the protein content. Indeed the firmness of the chosen fat blend must also be considered, as must also local water hardness where certain hydrocolloids are selected.

For a 60% fat spread, distilled saturated monoglyceride from a base of either rapeseed or soya at a dosage level of 0.4% will give the necessary stability and droplet size required. For 40% fat spread without protein, 0.5% of distilled unsaturated monoglyceride from vegetable base is recommended, whereas if protein is present, then either 0.5% of rapeseed or soya-based distilled saturated monoglyceride, or a combination of 0.5% of palm based distilled saturated monoglyceride or 0.5% soya based distilled saturated monoglycerides and 0.1%-0.2% PGPR is recommended. For 20% fat spreads without protein either 1.0% distilled unsaturated monoglycerides, or a combination of 0.5% distilled unsaturated monoglycerides with 0.4% PGPR is recommended. Finally, for 20% fat spreads with protein, the recommended combination is 0.6% distilled unsaturated monoglycerides with 0.4% PGPR. These combinations are fairly typical, and will of course be optimised according to best practice.

In low-fat spread applications, which have a high water and protein content, polyglycerol polyricinoleate (PGPR) can be used to great effect. It possesses exceptional water binding properties through which it secures the necessary emulsion stability and water dispersion. Under European rules, according to EC directive 95/2/EC, PGPR (E476) is allowed for use in low-fat spread applications with 41% fat or less in a maximum dosage of 0.4%.

For the reduced fat systems that are used for frying, a range of different emulsifiers is suggested such that the emulsion itself can be made to stability levels as those above. Here the water droplet size is vigorously controlled to hinder the spatter that typically plagues this application. The fat content of these systems is more readily termed reduced as it is about 60–70%, but even here good frying results are gained. Going to lower fat contents for frying is not really feasible. The emulsifiers therefore for the 60% fat frying systems are generally combinations. It is difficult for one single emulsifier to cover all the demands alone. Hence, combinations of citric acid esters with saturated distilled monoglycerides, or other vegetable based emulsifiers together with lecithin are generally recommended.

When referring to the reduced and low fat systems generally, it is important to account for texture and control the crystallisation of the fat phase. β' is the stable fat crystal form for desirable mouthfeel texture as opposed to the β, but the tendency is for fat crystals to proceed to the β form. As said earlier, this tendency can be hindered or indeed prevented, within the products shelf life by addition of sorbitan

tristearate (STS). Due to its irregular shape, STS prevents the 90° rotation of the fat molecules towards the β form. Typically, STS is added at around 0.5%.

Another problem facing the low-fat systems is the prospect of oiling out, a phenomenon, which is prevented or at least reduced by stabilising the crystal lattice at higher than ambient temperatures. This is achieved by use of a high-melting stabiliser, where the dosage is basically governed by the degree of oil separation to be prevented. Higher oiling out tendency therefore requires a higher dosage of the high-melting stabiliser. Here, a vegetable fat/emulsifier blend is recommended with a dosage level ranging from 1% to 2%.

The water phase of the low-fat systems requires special attention, as the use of emulsifiers themselves is insufficient to achieve the stability required. This is true not just because of the amount of water present, but also the incorporation of proteins, be they skimmed milk proteins (casein) or whey based. The action of the proteins is to form a looser, more open emulsion that improves flavour release. A down side effect is observed, whereby there is reduction of emulsion stability. Hence the need for other stabilisers: hydrocolloids. Much has been made of gelatine in this application due to its very specific melting properties. However, the modern trend has been to find gelatine alternatives and the ones of prime choice are pectin and alginate, used either alone, or in combination.

When controlling the water phase with the hydrocolloids, the aim, indeed the optimum, is to make sure the water phase and the fat phase have a similar viscosity when the low fat spread product is processed. If this is achieved, then a stable, homogeneous low-fat spread product is achieved without water separation (syneresis). Achieving similar viscosity of both water and fat phase is possible by varying the hydrocolloid type and dosage as well as the protein type and dosage together with using a softer fat blend. However, solutions based on softer fat blends (less saturated) can be problematic in regions where there is inadequate temperature control at higher ambient conditions. This is because the low fat spread must be sufficiently firm to be acceptable and spreadable, and have a good mouthfeel. Investigation into the effect of stabilising reduced TAG based water-in-oil emulsion systems has shown that a multidisciplinary approach is required to understand the impact and relationship of minor ingredients on physical and sensory properties of the emulsion (Wassell 2013).

13.10 Product Spoilage

Shelf life issues regarding spoilage of the margarine or spread product is important to consider. Two types of spoilage occur, that due to microbiological contamination and that due to chemical rancidity, i.e. fat oxidation.

Yeasts, bacteria or moulds are responsible for the microbiological contamination of margarines or low fat products. These species are generally unable to grow in fat and oil systems. Therefore contamination in the margarine type products occurs through growth of these species in the water droplets within and on the product's

surface. Microbiological spoilage is influenced by water droplet size, protein content, salt content, and pH.

The smaller the water droplet size, the less attractive the environment for the micro-organisms since less nutrients are available to them. Similarly, the smaller the water droplet size the greater is the proportional chance of more sterile water droplets than actual micro-organisms. Hence, small water droplet size increases the shelf life of the product, and this is a side function of what the emulsifiers are able to do on grounds mainly of texture and stability. By small, it is meant that the average droplet size in margarine is 4 ± 5 μm with a range from 1 to 20 μm. When the droplet size is less than 10 μm, it is doubtful that these restrictive environments will allow micro-organism growth (Delamarre and Batt 1999). In reality good manufacturing practice (GMP) must come into play, because the margarine and spreads (water-in-oil) industry is generally regarded as low risk, sometimes larger size droplets are found because of the acceptable trade-off with required flavour release.

Protein will act as nutrients for the micro-organisms unless salt and pH levels are addressed. For the same margarine with 16% water, addition of 1% salt overall will inhibit the growth of many micro-organisms whereas addition of 2% salt will prohibit almost all. It should be noted that addition of low levels of salt, around 0.1–0.2% overall may actually enhance the growth of the micro-organisms. It is worth noting that it is the salt content in the water phase that is important, and thus as a rough guide, a margarine with 16% water and 1% salt overall results in a 6% salt content in the water phase alone.

pH is similarly an issue, and generally low pH values inhibit more micro-organisms that higher pH values, i.e. around pH 4.0–4.5 micro-organism growth is retarded. Higher pH values of 5.5–6.0 enhance growth. Adherence to GMP will avoid pathogenic contamination.

Chemical rancidity or oxidation occurs in the fat phase and is caused by a reaction between the fat and oxygen. The reaction takes place at the double bonds of the fatty acids, forming peroxides, aldehydes and ketones, etc. The composition of the water phase is important since the oxidation process begins at the interface between the water and fat phases. Once started, oxidation proceeds quickly. Factors influencing the oxidation rate include the composition of the fat blend, oxygen availability, metal ions, salt, pH, water droplet size, and light.

The more double bonds present in a fatty acid the quicker it will oxidise. Stearic acid is 10 times more stable towards oxidation than oleic acid; 100 times more stable that linoleic acid, and 1000 time more stable that linolenic acid when kept at the same temperature. Also the greater the concentration of liquid oil in the fat blend the more prone it will be to oxidation during its shelf life. Generally the following oils oxidise most easily in the following order of diminution: Safflower oil, soya bean oil, rape-seed oil, sunflower oil, corn oil, cotton seed oil, and ground nut oil.

Atmospheric oxygen should be limited in its contact with the oils, often practically achieved by blanketing the processing tanks with nitrogen. Metal ions can also increase the tendency for oxidation, copper ions in particular and therefore any piping and tubing in margarine plants should not be made of copper or copper alloys. The use of sequestrants in the water phase, and water softening capability will also

help to minimise effects of oxidation. Salt will help to catalyse the oxidation process, such that more salt is equivalent with faster oxidation. Similarly low pH values will aid the oxidation steps (4.0–4.5), and higher values (5.5–6.0) will reduce the tendency. Hence, large quantities of salt should not be used at low pH. Small water droplets lead to a large interfacial surface area between the water phase and the fat phase increasing the rate of oxidation onset.

As can be seen from the three latter examples of salt, pH and water droplet size, they are in direct contrast to the conditions demanded to stop micro-organism attacks!

Light, especially UV will strongly catalyse the oxidation process, and of course the product should be stored under cool, refrigerated conditions.

Given that the contamination by micro-organisms and the texture and mouthfeel qualities demanded of the margarine and spread products are at odds with the conditions required to minimise oxidation, the oxidative problems are solved by adding in a range of dedicated antioxidant materials. These may be the well-known phenolic antioxidants of BHA, and BHT, etc. but these are gradually being replaced by vitamin based products such as ascorbyl palmitate or even natural extracts such as rosemary extract.

13.11 Summary

The traditional margarine of 80% fat content is a very stable product and does not require a great deal of emulsifiers to hold the structure demanded be they mono- or diglycerides, lecithin or citric or lactic acid esters of the monoglycerides over and above any proteins that might also be present. Performance of industrial margarines can depend very much on the emulsifier system. As the fat content is reduced to 60% and below, the presence of emulsifiers is a pre-requisite to hold the emulsion stable, homogenous and still give the product the functionality the application demands. At fat contents of 40% other ingredients (hydrocolloids) are required to further stabilise the water phase and these work in cooperation with the emulsifiers. When dealing with these low fat content products it is important to understand the nature of the application of the product such that the correct emulsifiers can be chosen for the particular process and application (Wassell 2013). Today, and looking forward, we will likely see more margarine and spread emulsion products manufactured first with lower total saturated fatty acids, and second with lower sodium content. Likely, the former helped by greater supply and utilisation of both GMO and non-GMO high oleic vegetable oils (Ramli et al. 2008).

To maintain and prolong shelf life of the product antioxidants are usually added to hinder onset of rancidity that will naturally occur. Micro-organism contamination is usually dealt with by making the structure of the margarine, shortenings and spreads unattractive for them. These conditions happen to coincide with the desired conditions for optimal functionality, mouthfeel, and textural properties of the product, which do not remove the challenge to the developer, but do mitigate them significantly.

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Chapter 14
Application of Emulsifiers to Reduce Fat and Enhance Nutritional Quality

Matt Golding and Eddie Pelan

14.1 Introduction

At a time when both malnutrition and obesity are increasingly becoming global issues, it is perhaps unsurprising that health, nutrition and weight management are the current main consumer trends within the food industry. As a consequence of these trends, innovation within this sector is being driven by the need to reduce perceived "bad" ingredients: (saturated/trans) fat, salt and sugar, whilst attempting at the same time to fortify foods with nutritional actives, such as minerals, vitamins and antioxidants, all in support of a healthier lifestyle. The market for reduced fat/reduced calorie products is highly lucrative. In the UK alone, this market segment was worth GBP1875m in 2004, up from GBP1372m in 2000. In 2005, sales are expected to reach GBP1975m. However, it should be stated that in moving towards healthier, more nutritious products, the demanding consumer still expects that the quality of the particular food in question is not compromised in terms of overall sensory performance (appearance, texture, flavour).

The use of emulsifiers as a structuring tool for fat reduction and/or nutritional enhancement is exemplified in many food product systems. Some examples of emulsifier applications for fat reduction, such as fat structuring in homogenised creams and ice creams, are not necessarily new innovations. However, there are also more recent developments, such as the use of emulsifier mesophase technology, which has found application in products such as zero fat ice creams and spreads.

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This chapter reviews some of these diverse applications, both old and new, aiming to show the versatility of emulsifiers when in food formulations for the purpose of fat reduction and nutritional enhancement. The term emulsifier in this instance refers specifically to (non-protein) molecules derived from fatty acids, such as lecithins, monoglycerides and their derivatives. It aims to examine the contribution of emulsifiers in improving product structural design as a means of reducing or eliminating (saturated) fat from food systems, whilst attempting to maintain the quality of the food product. It also aims to explore the use of emulsifiers as delivery mechanisms for nutritional enhancement of foods.

14.2 The Intrinsic Nutritional Value of Emulsifiers

It is well established that the diversity of functionality provided by emulsifiers in the design of foods can be utilised by food manufacturers to deliver an equally diverse set of product benefits. In this way, technical properties such as emulsification, demulsification, foaming, wetting and specific interactions with other food ingredients can be used to generate improvements in product aspects such as processability, physical stability, sensory performance and product shelf life (Kralova and Sjoblom 2009).

Another application area of particular research and commercial interest is the use of these materials in the design of food products with improved nutritional profiles or with added health and wellness benefits. As of themselves, the consideration of the intrinsic nutritional value of lipid-derived emulsifiers remains a highly debatable topic. Various studies have investigated whether the consumption of natural surfactants has particular physiological benefits.

Notably, the nutritional value of phospholipids, as derived from a variety of sources, has been widely studied. Detailed reviews of this area are provided by a number of researchers, including Castro-Gomez and co-authors (2015) and Kullenberg and co-authors (2012), who present a comprehensive summary of the health benefits associated with dietary phospholipid intake. Without expanding into too much detail, the impact of dietary phospholipids on the following physiological conditions has been evaluated: cancer (prevention and treatment); neurological disorders and cognitive impairment; lipid profile, cholesterol and cardiovascular risk; inflammation and related diseases; immunology; infections; and hepathopathies. Many of these studies reported positive outcomes associated with phospholipid intake when carried out as part of clinical trials, notably the particular influence of the milk fat phospho- and sphingolipid system, which has been reported as demonstrating multiple health benefits (Dewettinck et al. 2008).

Another class of natural surfactants, the saponins, has also come under increasing scrutiny in regards to potential health promoting attributes. Saponins are be defined as a group of compounds comparison triterpenoid or steroidal aglycones linked to oligosaccharide groups. The aglycone moiety is predominantly hydrophobic and when combined with the hydrophilic sugar groups imparts amphiphilic

characteristics to the molecule. Saponin-rich plants, such as tea, quinoa and quillaja have been recognised for many years for their utilisation as naturally derived detergent materials, and isolated and purified saponins have been increasingly considered for foaming and emulsifying applications across a range of industry areas including food (Moses et al. 2014). Whilst less well studied compared to phospholipids, the health and wellness benefits associated with consumption of saponin still shows remarkable diversity, being demonstrated as possessing immunostimulatory, hypocholesterolemic, anti-carcinogenic, anti-inflammatory/antioxidant and antimicrobial properties.

In contrast, synthetically derived emulsifiers are less well considered in terms of actual health benefits arising from their consumption. This may be due to lack of study in this area, possibly as a consequence that these materials are not biologically derived. However, it is worth recognising that, at least in the case of monoglycerides, a biological equivalence does exist as part of the lipid digestion process, in which dietary triglycerides are hydrolysed through the action of digestive lipases to component fatty acids and monoglycerides fractions, and that the polar nature of biological monoglycerides forms an important aspect of fatty acid uptake through the formation of the mixed micelle system. Digestive and commercial monoglycerides (most notably monolaurin) have also been shown to have powerful antimicrobial, antiviral and antifungal properties (Schlievert and Peterson 2012). Whilst digestive monoglycerides have been indicated as having a role in controlling the microbial environment within the GI tract (having particular importance during infancy (Heine et al. 1998)), the antimicrobial function of commercial monoglycerides has also been explored in both food products and processes (Thormar and Hilmarsson 2012; Hauerlandova et al. 2014).

As indicated, for other commercial polar lipid emulsifier derivatives there is less evidence of specific health or wellness properties. Lack of nutritional value coupled with the synthetic nature (and chemical-sounding nomenclature) of these materials may account for their poor consumer perception in products. There is also some consideration that ingestion of these materials may have possible negative health consequences (Csaki 2011; Lerner and Matthias 2015), although it seems reasonable to indicate that all permitted emulsifiers have undergone extensive toxicological screening and have in many cases been used extensively in food products for decades with no chronic adverse effects being reported.

However, in terms of imparting health and wellness benefits to food products, it can be argued that the value of both naturally and synthetically derived emulsifiers lies not in any specific intrinsic nutritional property, but in the broader spectrum of technical functions that enable the nutritional value of other micro or macronutrients to be effectively incorporated into food products, thereby enhancing the overall nutritional profile of the food. In this respect, the technical functionality may range from stabilising or protecting the relevant nutrients during processing and product shelf life, through to ensuring appropriate bioavailability and bioefficacy of nutrients during digestion. This chapter will aim to provide pertinent examples of how the functionality of emulsifiers can employed during food formulation and manufacture to enable nutritional enhancement of food products.

14.3 Homogenised Dairy and Non-dairy Whipping Creams

Homogenised whipping creams have been produced commercially for over four decades. They are specifically designed to imitate the organoleptic properties of non-homogenised dairy creams for the main application properties of cooking, baking, pouring and whipping. In the particular case of whipping cream formulations, some of the main advantages presented by homogenised dairy and non-dairy creams compared to non-homogenised dairy analogues are improved shelf life (through UHT treatment), more robust whipping properties (less chance of buttering), and especially reduced fat level (reduced from typically 30–40% in dairy systems to <20% in non-dairy systems). Whilst the natural composition of dairy cream lends itself to providing good whipping properties above a certain fat level, the challenge with homogenised dairy and non-dairy whipping creams is to design an emulsion system with comparable whipping and sensory performance at these greatly reduced fat concentrations.

Dairy whipping cream is seen as an indulgent product, understandable considering the relatively high fat content. Homogenised non-dairy and dairy creams aim to target this high fat content offering lower calorie alternatives. A comparison between the caloric content of some dairy and non-dairy whipping creams is given in Fig. 14.1.

Whipping creams are aerated emulsions with overruns typically ranging from 100% to 300%. Whipped creams should also possess good stand-up properties (i.e.

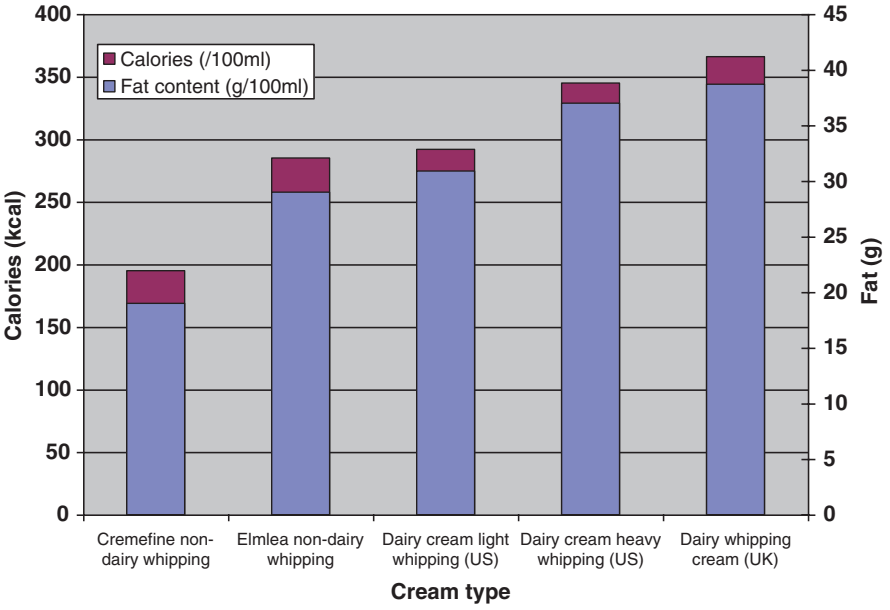

Fig. 14.1 Examples of caloric and fat contents of some non-dairy and dairy cream samples

the foam structure should be self-supporting and not flow). Although foam lifetime of whipped creams is not intended to be more than a few days, there should not be any visible ripening of the foam structure during the lifetime of the product. The mechanism by which a stable foam structure can be generated by whipping of dairy cream has been of considerable academic and commercial interest for a number of years (Flack 1985; Bruhn and Bruhn 1988; Goff 1997; Leser and Michel 1999; van Aken 2001), and is discussed at greater length in Chap. 7 of this book.

In order to demonstrate how the use of emulsifiers can contribute to the development of a low-fat whipping cream, we need to review the mechanism by which whipped structures can be prepared. An elegant model for the development of the whipped cream structure is provided by Besner and Kessler (1998), who described the mechanism as occurring in three stages during the whipping process (Fig. 14.2):

(a) Protein adsorption at the air water interface to provide initial foam stability. Milk proteins are generally present in both dairy and non-dairy cream formulations. In the specific case of dairy cream, most of the casein and whey protein is present in the continuous phase of the emulsion is not adsorbed at the oil-water interface (Needs and Huitson 1991), forming a foam. At this stage, overrun is still low and the cream possesses no stand-up properties.
(b) Adsorption of fat globules to the air-water interface. During the whipping process, the weak milk fat globule membrane allows fat droplets to adsorb to the surface of protein stabilised air-bubbles. This is possibly due to the rupture of the MFGM during the shearing process, which allows wetting and partial spreading of fat droplets on contact with the bubble surfaces. The formation of the globule-coated interface is more effective at preventing bubble coalescence than a milk-protein stabilised interface.
(c) Fat globule adsorption to the bubble surfaces facilitates globule aggregation in the continuous phase. Droplet aggregation and subsequent formation of a fat globule network is required to prevent drainage of the stabilised foams and provide body/stand-up to the whipped cream. The shearing process leads to partial coalescence of fat droplets, an irreversible aggregation process in which

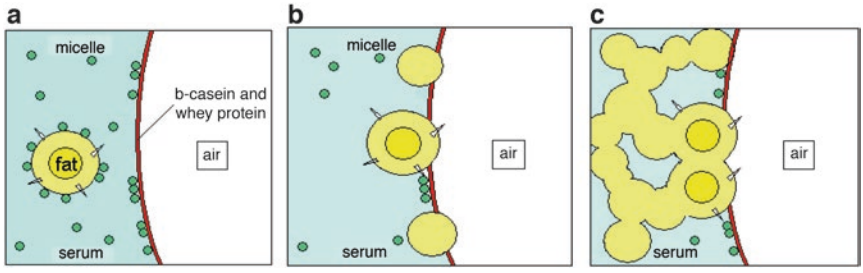

Fig. 14.2 Highly schematic representation of structure development in dairy whipping creams. (**a**) Initial stabilisation of air phase by adsorbed proteins, (**b**) secondary stabilisation of air phase by adsorption of fat globules, (**c**) development of partially coalesced fat network in the continuous phase

fat wetting between two or more droplets can take place (Boode and Walstra 1993; Vanapalli and Coupland 2001). The presence of solid fat within the emulsion prevents full coalescence from taking place, so droplets partially maintain their integrity, hence the name (Boode et al. 1993) (Fig. 14.3).

A good whipped cream structure requires both fat globule adsorption to the surface of the bubbles in the foam, and the generation of an aggregated fat network in the bulk. This structure has been visualised by a number of authors (Buchheim 1991; Brooker 1993) and is described in more detail in Chap. 7.

Non-dairy whipping creams and homogenised low-fat dairy whipping creams are formulated and processed to provide structuring according to this particular mechanism of whipping. A typical non-dairy whipping cream composition is given in Table 14.1.

For non-dairy creams butterfat is replaced by vegetable fat(s). These are commonly high lauric fats such as coconut or palm kernel oil, which provide the required solid fat content at whipping temperatures, but which melt at in-mouth temperatures (thus providing the desired oral response). As stated previously, non-dairy whipping creams can provide whipped structures with acceptable organoleptic properties at almost half the fat content of a conventional dairy whipping cream.

The milk solids nonfat component (MSNF) is usually either skimmed or butter milk powder, which is added, in part, to provide a dairy flavour to the cream. However, the MSNF also contains the milk proteins: casein and whey. Unlike dairy creams, non-dairy creams require a homogenisation step to form a stable emulsion. Milk proteins are important to the formulation, as they provide the initial stability to the emulsion on homogenisation. Droplet size for homogenised dairy and non-dairy

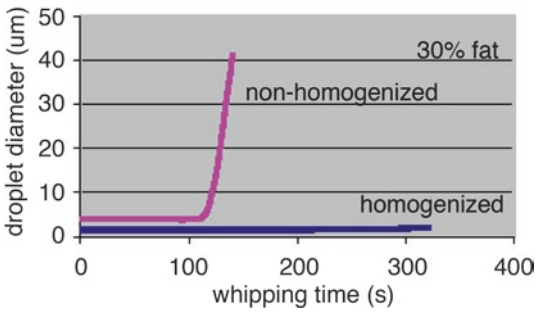

Fig. 14.3 Change in emulsion droplet diameter as a function of whipping time for 30% homogenised and non-homogenised whipping creams. (Adapted from Besner H, Kessler HG, Milchwissenschaft 53 (12): 682–686, 1998)

Table 14.1 Typical non-dairy whipped cream composition

Composition	Amount
Fat	20–30%
MSNF	3–6%
Added sugars	5%
Stabilisers	0.05–0.2%
Emulsifiers	0.05–0.6%

creams is typically 1 μm or less, which is at least a quarter of that usually encountered for non-homogenised dairy creams. The reduction in droplet size also corresponds to a significant increase in specific fat surface area, which may account for the fact that less fat is required to provide a stable foam structure in the case of homogenised cream.

Protein is essential to provide a stable emulsion during preparation of the cream. However, the adsorbed protein layer prevents adsorption of globules to the air-water interface, and provides effective stability against partial coalescence during the whipping process. In order to achieve the functionality required to generate appropriate whipped structures, emulsifiers are included in the formulation. These have little or no effect on the stabilisation of the emulsion during homogenisation during processing, since at the temperatures applied during homogenisation (typically 80 °C) there is less of a difference in interfacial tension between the emulsifier and the protein. However, on cooling, an interfacial tension gradient opens between the protein and the emulsifier, with the result that the emulsifier displaces the protein from the interface. Displacement of adsorbed protein and replacement by emulsifier interfacial layers has a significant impact on the stability and functional properties of emulsion systems. Consequently, the displacement of protein by emulsifiers from interfaces has been the subject of considerable academic attention in recent years (Segall and Goff 1999; Stanley et al. 1996; Tual et al. 2005, 2006).

In the particular case of non-dairy whipping creams, addition of emulsifiers has been shown to facilitate adsorption of fat globules to the air-water interface during whipping. This appears to be a common effect to most emulsifier systems, and therefore most emulsifier types will contribute to the interfacial stabilisation of the foam. There is some speculation as to why the presence of an emulsifier layer on the droplet interface should promote adsorption to the bubble surface. However, it may be related to the fact that regions on the fat globule surface where displacement has taken place are more interfacially active than the protein layers adsorbed to the bubble surface during the beginning of the whipping process.

Consequently, during collisions with the bubble surfaces during shearing, fat globules become preferentially adsorbed to the air-water interface. Increasing emulsifier concentration will result in higher surface coverage of the emulsifier at the droplet interface and will therefore increasing the potential for a droplet to adsorb to a bubble surface during whipping. This particular aspect of the whipping process is used to great effect in the stabilisation of aerosol creams. Here, emulsifiers are used specifically to promote the adsorption of fat globules to the air-water interface where they provide excellent stability to the foam. Fat structuring in the bulk phase is not necessary since foam structure is derived from the high overrun produced by the aerosol.

For homogenised whipping creams, addition of emulsifiers also promotes fat structuring during the whipping process, which is essential for providing rigidity to the cream. Type and concentration of emulsifier can have a significant impact on emulsion structuring properties. In short, it can be stated that displacement of protein from the oil-water interface by particular emulsifiers can create active sites on the droplet surface which can result in droplet aggregation under shear (see Chap. 7

for more information). The nature of the droplet aggregation is understood to be dependent on the type and concentration of the emulsifier systems used (Krog and Larsson 1992).

Whilst it is certainly true that partial coalescence does take place for particular formulations of homogenised whipping creams, it is not necessarily the only type of aggregation observed during the whipping process. Under certain formulation conditions it is possible to design emulsions that form network structures through interfacial aggregation, as opposed to partial coalescence. In these circumstances, there is no rupturing of the interfacial layer. Both partial coalescence and interfacial aggregation result in the build-up of a fat network (similar to the processes taking place for dairy whipping cream), which increases the stand-up properties of the cream.

Whilst most food grade emulsifiers have the ability to displace protein from the interface of emulsion droplets, it is important to note that the composition and nature of the interface can vary significantly according to the specific emulsifier or emulsifiers used. Even emulsifiers with similar structures and HLB values can provide very different interfacial (and thus whipping) properties. As such, there are no definitive guidelines for which emulsifiers can provide acceptable whipping properties, although it is understood that particular emulsifiers are more effective at promoting fat adsorption to the air interface, whilst others are more effective at structuring the emulsion under shear. Often a combination of emulsifiers provides the most effective whipping properties in terms of aeration and fat structuring.

The composition of the oil-water interface is the main determining factor for how the emulsion behaves on whipping. Choosing the most appropriate emulsifier system for a non-dairy cream formulation and optimising its concentration and processing conditions will determine the functionality of the cream. Optimising emulsion droplet functionality is critical in determining whether a cream will be stable under storage conditions yet has acceptable whipping properties when aerated.

The current challenges facing the non-dairy creams industry are the ability to produce cream with acceptable structuring properties whilst continuing to lower the fat content of the cream. Whipping creams with less than 20% fat are now commercially available. Whilst there are a number of other structuring routes that can be used to provide whipped structures at even lower fat levels, the further reduction in fat will eventually lead to an unacceptable loss of sensory performance.

Additionally, removal of saturated triglycerides from formulations and replacing them with unsaturated triglycerides, whilst maintaining the textural and flavour properties associated with whipped cream. Solid fat is a particular requirement for providing acceptable stand-up properties of whipped creams, both dairy and non-dairy. Manufacturing whipping creams with high levels of unsaturated oils which can be aerated and possess good structure is not a trivial exercise.

14.4 Reduced and Low Fat Ice Cream

Depending on which historical perspective is correct, ice cream has been consumed as an indulgent treat for between 300 and 700 years. At its most basic, ice cream can be described as an aerated frozen foam containing milk, cream, sugar with flavouring added (still most commonly vanilla). However, due to the consistent popularity of ice cream as a foodstuff (14.4 billion litres were sold globally in 2001), there are now many choices in today's ice cream market in order to suit a wide variety of consumer tastes. In order to provide some explanation as to the numerous ice cream formats available for purchase, the US FDA has set up standards of identity to characterise ice creams according to formulation. Whilst these are not necessarily applied globally, they can provide useful information regarding consumer trends in the consumption of ice cream. A summary of the FDA classification of ice cream is as follows:

- **Ice cream**, an aerated, frozen food made from a mixture of dairy products, containing at least 10% (milk) fat.
  - ***Superpremium*** ice cream tends to have very low overrun and high fat content, and the manufacturer uses the best quality ingredients.
  - ***Premium*** ice cream tends to have low overrun and higher fat content than regular ice cream, and the manufacturer uses higher quality ingredients.
  - ***Regular*** ice cream meets the overrun required for the federal ice cream standard.
  - ***Economy*** ice cream meets required overrun and generally sells for a lower price than regular ice cream.
- **Reduced fat** ice cream contains at least 25% less total fat than the referenced product (either an average of leading brands, or the company's own brand).
- **Light** ice cream contains at least 50% less total fat or 33% fewer calories than the referenced product (the average of leading regional or national brands).
- **Lowfat** ice cream contains a maximum of 3 g of total fat per serving (125 mL).
- **Nonfat** ice cream contains less than 0.5 g of total fat per serving.

The current consumer trends within ice cream present something of a paradox. Whilst consumers are generally becoming more health conscious about what they eat, the highest market segments in ice cream at the moment are the premium and super-premium brands of ice cream, which can contain anything between 10% and 20% fat (Fig. 14.4). At these high fat levels (usually in the absence of added emulsifiers), there is a dominant contribution of the fat phase to the sensory properties of the ice cream (creamy texture and flavour) as well as to the meltdown stability. Unfortunately, there is also a significant contribution to the caloric content as well!

To a degree this is accepted: ice cream has always been perceived as an indulgent product—with fat level as an indicator as to the quality of the product. Consequently, lowering of the fat content within the formulation is often accompanied by a perceived reduction in sensory quality of the ice cream. The relationship between

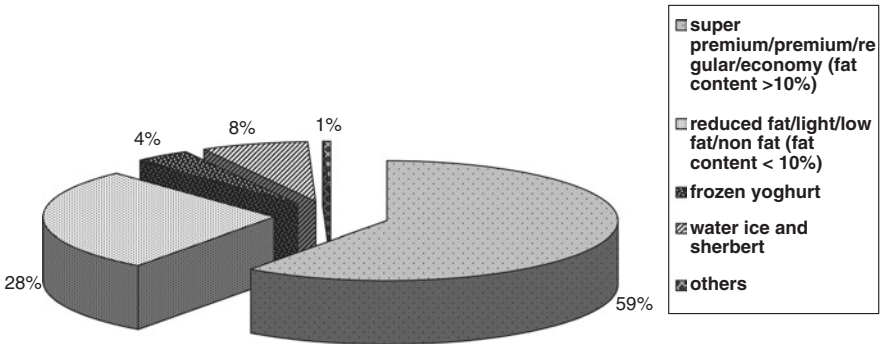

Fig. 14.4 Market segments for 2004 US Ice Cream market showing, amongst others, market share for high (>10%) and low (<10%) fat ice cream products. US Ice Cream market was estimated at 1.6 billion US gallons in 2004

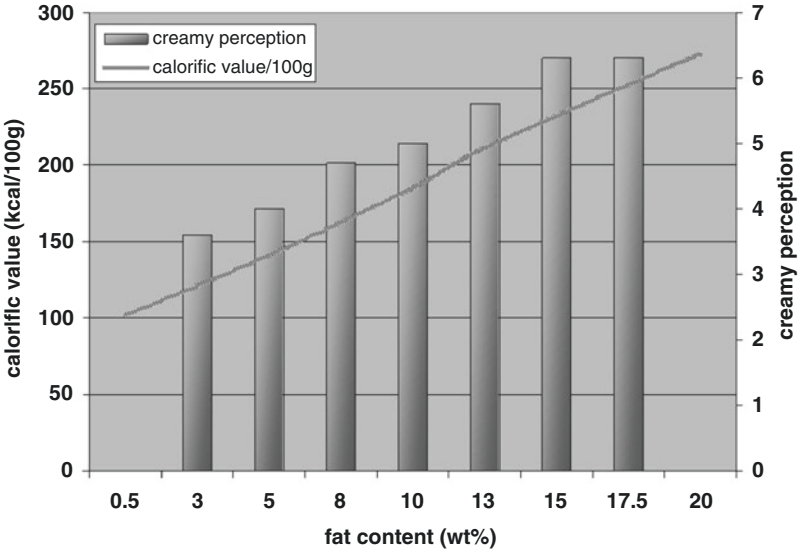

Fig. 14.5 Graph showing the relationship between fat content in ice cream, calorific content and perceived creamy texture. Ice creams were consistent in formulation and did not contain emulsifiers

calorific content, as supplied by fat, and the perceived creaminess of the ice cream (in the absence of emulsifiers) is given in Fig. 14.5. However, it is possible to formulate ice creams with a lower fat content in which the sensory properties of the ice cream are not compromised by the reduction in fat.

One route by which the quality of lower fat ice creams can be improved is through the inclusion of low concentrations (0.1–0.5%) of emulsifiers to the ice cream mix. The use of emulsifiers in ice cream formulations is not particularly new,

Table 14.2 Ingredient breakdown of a typical regular ice-cream

Ingredient	Amount (wt%)
Fat	5–15
Milk protein	4–5
Lactose	5–7
Other sugars	12–16
Stabilisers	0–0.5
Emulsifiers	0–0.5
Total solids	28–40
Water	60–72

and its earliest application dates back to the 1940s. As with whipping creams, emulsifiers are added to improve the functionality of the fat, such that the fat becomes an active component in the development of the ice cream structure. This can lead to improved product attributes, such as dryness upon extrusion, improved air phase stability, improved meltdown resistance and improved sensory performance of the ice cream, especially for lower fat formulations). A typical ice cream mix is given in Table 14.2.

Processing of ice cream requires the mix to be pasteurised, homogenised and aged prior to freezing. Prior to homogenisation, water soluble ingredients such as stabilisers, sugars and proteins are dispersed in the aqueous phase. Any oil soluble components are dispersed in the oil phase before the two phases are mixed. Emulsifiers used in the ice cream industry are limited by legislation and are predominantly monoglycerides, and to a lesser extent polysorbates. Monoglycerides, being of low HLB are generally dispersed in the oil phase, whilst the polysorbates being of higher HLB are placed in the aqueous phase.

After homogenisation and ageing the mix is transferred to the ice cream freezer. An ice cream freezer is essentially a scraped surface heat exchanger, operating at approximately −20 °C into which air is channelled at a pressure of 2 bar. The low temperatures on the surface of the heat exchanger barrel form ice crystals, which are scraped into the ice cream mix. In addition, the high shear forces applied within the freezer assists in aeration of the ice cream. This combination of high shear and low temperature creates the frozen foam ice cream microstructure. The ice cream is then extruded from the freezer before being hardened to at least −30 °C. Storage of ice cream is generally maintained at −18 °C, although some formulations are designed to be stored at temperatures as warm as −10 °C. Volume fractions of the various phases are given in Table 14.3 for different ice cream formats, whilst the distribution of these phases in a typical ice cream microstructure is shown by scanning electron microscopy in Fig. 14.6.

The mechanism by which addition emulsifiers can influence the microstructural properties is in some respects similar to the effects observed for homogenised whipping creams. As with whipping creams, emulsifiers are added in order to displace protein from the interface of the fat droplets. This takes place during the ageing

Table 14.3 Typical phase volumes of ice cream components

Phase	Low fat ice cream (%)	Regular ice cream (%)	Premium ice cream (%)
Fat	1	5	10
Air	48	50	35
Ice	31	30	25
Matrix	20	15	30

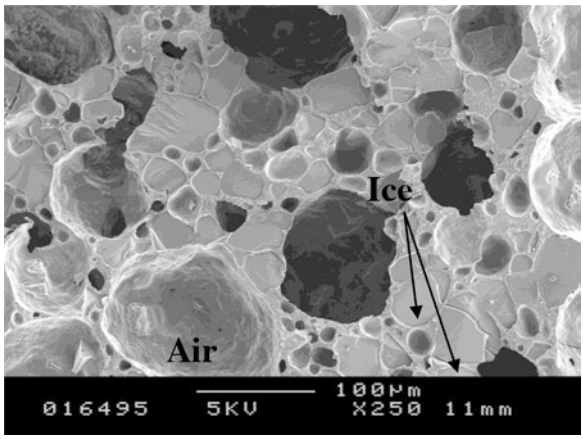

Fig. 14.6 Scanning electron micrograph of ice cream microstructure showing air bubbles, ice crystals and surrounding matrix

process after homogenisation. The presence of the emulsifier on the surface of the emulsion droplets facilitates the adsorption of the droplets to the air-water interface during freezing (again the analogy with whipping cream systems can be drawn, since droplets stabilised purely by protein do not undergo adsorption to the surface of bubbles).

Several studies have been carried out to better understand the mechanism by which fat globules containing emulsifiers can adhere to the air-water interface. Whilst the exact mechanism is still the subject of some speculation, it has been recently shown by Zhang and Goff (2005) that the process is sensitive to both the type and concentration of both emulsifier and protein present during the freezing process. In the case of the emulsifier, this is in part influenced by the efficacy by which specific emulsifiers can displace protein from the interface—the more droplet surface coverage by the emulsifier, the greater the potential for adsorption to the surface of a bubble.

Pelan et al. (1997) showed that displacement from the interface varied according to the emulsifier used (Fig. 14.7), and that for the commonly used ice cream emulsifiers, displacement increased in the order:

Saturated monoglycerides < *unsaturated monoglycerides* < *polysorbates*

Differences in displacement between the two types of monoglyceride has in the past been attributed to the structural arrangement of the two emulsifiers at the oil-water interface. However, it may also be due to the fact that saturated monoglycerides are able to nucleate fat crystals on cooling and may therefore become trapped

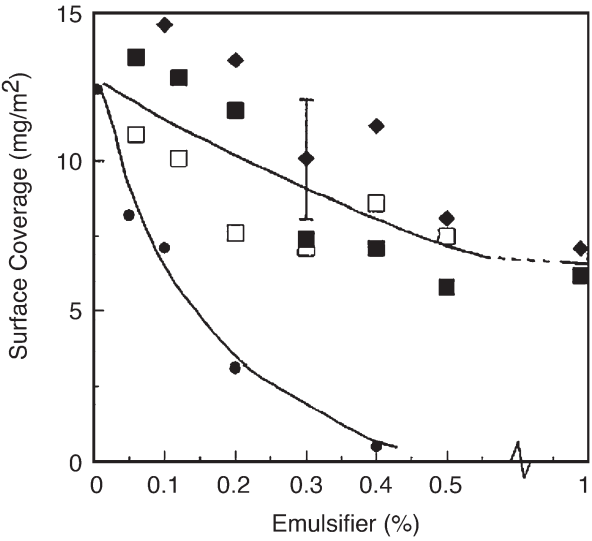

Fig. 14.7 Change in protein loading for ice cream mixes (12% fat, 13% SMP, 15% sucrose) as a function of emulsifier type and concentration. ● Tween 60; □ Unsaturated monoglyceride; ■ Saturated monoglycerides; ◆ Glycerol monopalmitate. (With permission, from Pelan et al. 1997)

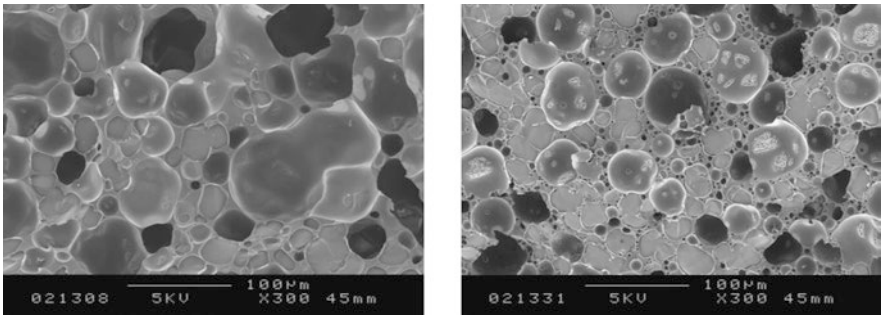

Fig. 14.8 Scanning electron micrographs of zero fat ice creams. In the left hand image the air phase is stabilised purely by milk proteins present in the formulation. In the right hand image, 0.5% saturated monoglyceride has been added to the formulation prior to freezing

within the bulk of the oil droplet, rather than adsorbing to the interface. Unsaturated monoglycerides have a lower melting temperature than saturated monoglycerides and do not tend to act as nucleators. The fact that emulsion droplets containing unsaturated emulsifiers crystallise at a slower rate than those containing saturated emulsifiers may allow the unsaturated emulsifiers longer to adsorb to the oil-water interface, thereby displacing more protein at equivalent concentrations. Polysorbates are even more effective, since they are water-soluble and adsorb to the oil-water interface independently of the internal state of the oil droplets.

The ability for oil droplets containing emulsifiers to adsorb to the air-water interface has been shown to reduce bubble size during processing and improve bubble stability on storage (e.g., Fig. 14.8 for zero fat ice cream). As with whipped cream systems this is attributed to a Pickering type stabilisation mechanism, which prevents coalescence from taking place. Improvement to the fineness of structure

and the stability of the air phase are partly responsible for the improved textural attributes of ice cream containing emulsifiers compared to those without. Certainly, addition of emulsifiers and the adsorption of fat to the air interface helps inhibit loss of quality through air phase coarsening as a result of temperature cycling.

Inclusion of emulsifiers in the formulation also helps to promote structuring of the fat through partial coalescence, which takes place during the freezing process. Again, the displacement of protein from the oil-water interface weakens the fat droplets. Consequently, droplet collisions driven by the high shear forces in the ice cream freezer allow penetration of droplet interfaces by fat crystals leading to partial coalescence. Again, there are differences between emulsifier types in terms of the amount of aggregated fat generated in the freezer such that, for equivalent concentrations:

polysorbate 60 > *unsaturated monoglyceride* > *saturated monoglyceride*

Again, this is in part due to the relative amount of protein displaced by each type of emulsifier at the oil-water interface. However, in the specific case of saturated monoglycerides there is an optimum emulsifier concentration at which maximum fat aggregation can be achieved. If the saturated emulsifier level increases too much, droplets become more stable to aggregation. This is possibly due to the formation of a crystalline emulsifier layer on the surface of the droplets, which is thick enough to prevent fat penetration and wetting from taking place.

Whilst fat aggregation is unlikely to lead to the formation of extended fat networks in the same way as whipping cream (due to the lower fat content and presence of ice which disrupts the formation of network structures), localised fat structure formation does improve the meltdown resistance of ice cream. In this case, it is likely that small aggregates of fat inhibit drainage of liquid from the foam structure as the ice melts, holding the foam together for longer. Partial coalescence has been an accepted model for emulsifier-facilitated fat structuring in ice cream for many years now. However, it has recently been demonstrated that addition of emulsifiers can lead to other forms of fat droplet functionality, which can provide additional benefits in terms of quality improvements for reduced and low-fat ice cream.

Continued understanding of how emulsifiers add functionality to ice cream systems is necessary if the ice cream industry is to follow in the current trend of health and vitality. There is a constant need to improve the quality of low fat systems, and new challenges such as the replacement of saturated fat in formulations will require development of new approaches for how emulsifiers can continue to contribute to the improved structuring of ice creams.

14.4.1 Zero-Fat Ice Cream

The use of saturated monoglyceride emulsifiers has also been shown to provide a specific role in improving the sensorial attributes of ice cream systems in the absence of fat. Zero-fat ice cream is something of a niche market. Whilst it might be consid-

ered desirable that the overall calorific content of the ice cream is greatly reduced relative to ice cream containing fat, there is unfortunately a corresponding significant drop in product quality that is generally not acceptable for most consumers.

There are two potential routes for improving the sensory properties of zero fat ice cream. The first route uses direct replacement of fat with a non-fat substitute. Fat mimetics, such as microparticulated proteins, can provide limited sensory improvements, but these are expensive and quality enhancement is not particularly noticeable.

An alternative route is to provide sensory benefits through optimisation of the microstructure of the ice cream. It is known that ice cream quality is as much dependent on optimising microstructure, as it is about using high quality ingredients. It has been shown that even with the removal of fat from the composition, there are alternative, indirect formulation routes for improving the microstructure, and thus the organoleptic properties of the ice cream.

One particular formulation route that has been patented by Unilever and is currently used in zero fat formulations is the inclusion of a small amount of saturated monoglyceride into the ice cream mix. Although monoglyceride is classified as a fat/lipid on ingredients lists, the amounts used (typically 0.1–0.5%) are within legislation requirements for the ice cream to be labelled as zero fat.

The addition of monoglyceride in a fat-free ice cream mix has been shown to result in the formation of a considerably finer air phase structure compared with protein alone. Figure 14.8 compares micrographs of zero fat ice creams containing no added monoglyceride or 0.5% added saturated monoglyceride. The protein stabilised air phase shows bubbles typically 100 μm or larger, with some signs of coalescence also having taken place. In comparison, the ice cream containing the added emulsifier shows a bimodal distribution of stable air bubbles with a larger phase of typically 50 μm or less, and a high number of very small bubbles of <10 μm.

The observed bimodal distribution is suggestive that partial disproportion has taken place. The fact that bubbles of <10 μm can still be observed implies that this smaller fraction is resistant to complete disproportionation. These small, stable air bubbles are understood to provide a positive contribution to the organoleptic properties of the ice cream. They are stable melting at ambient temperatures, and may retain stability in the mouth, giving the perception of enhanced creaminess and reduced iciness.

Figure 14.8 indicates that the inclusion of a low concentration of monoglyceride can greatly improve the stability of the aerated structure within ice cream. Saturated monoglycerides display particular mesophase behaviour in aqueous media. At temperatures below the Krafft point, and for low concentrations they form β-crystals in water which do not have foaming capacity. However, in ice cream mixes the monoglyceride forms surface-active particulates. This is understood to be due to the formation of milk protein-monoglyceride liposome structures as a result of the homogenisation process. These are able to adsorb to the air-water interface during the freezing process. These particulates are able to provide considerably greater surface elasticity to the bubbles than protein alone (Fig. 14.9), providing effective resistance to coalescence and preventing complete disproportionation from taking place.

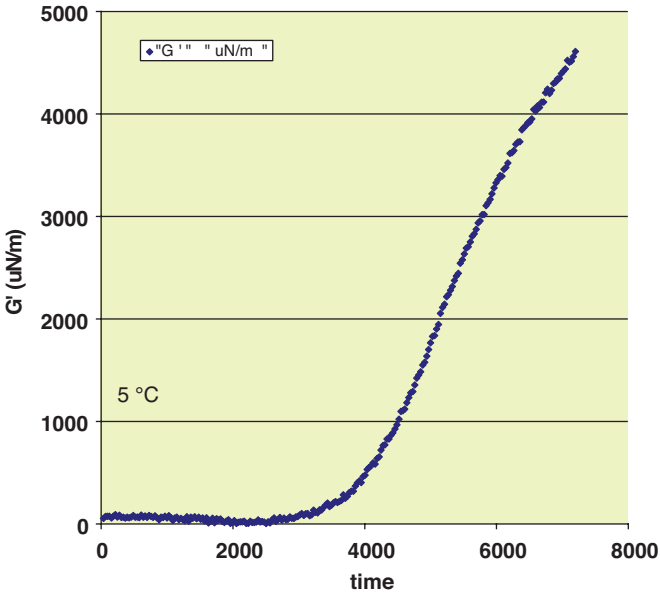

Fig. 14.9 Surface shear rheology (Camtel CIR-100 rheometer) of homogenised mixture of 2.5% sodium caseinate and 0.1% saturated monoglyceride at 5 °C (torque = 10,000 μrad, frequency = 3 Hz)

Saturated monoglycerides appear to be the most effective emulsifier for improving the stability of the air phase in zero fat ice cream. Unsaturated monoglycerides, for example, can also form particulates in the presence of milk proteins. These are known as cubosomes. However, it has been shown that whilst these are also surface active and can readily adsorb to the air-water interface, the surface elasticity of an interface stabilised by cubosomes is considerably lower than that of saturated liposomes. This may, in part, explain why unsaturated emulsifiers are less effective at providing foam stability in the absence of fat. Specific choice of emulsifier is ultimately limited by legislation. Whilst some alternative emulsifiers, such as polyglycerol esters of monoglycerides also show excellent foams stabilising properties in the absence of fat in a manner similar to that of saturated monoglycerides, these do not currently have clearance with the US and EU markets for application within ice cream formulations.

The use of monoglycerides to improve the sensory properties of zero-fat ice cream provides an effective example of how the relationship between ingredients processing and product microstructure can be manipulated to give improvements in the quality of low and zero-fat foods.

14.5 Margarine

Historical perspective.

Margarine was invented and patented by Mège Mouriès in 1869 as the result of a national competition from Emperor Napoleon during the economic crisis leading up to the Franco-Prussian war. Napoleon III needed a cheap butter substitute, which would feed his armies and remain edible after long journeys. Thus, the original advantage of margarine was that it offered a *high* calorific energy source that would be microbiologically stable for several months. Since then consumer demand and a changing world over the last 120 or so years have spurred margarine (spread) development to become one of the healthier (low fat) food types available today.

Mouriès theory was that butter fat was formed in the udder of the cow from its own fat and milk, so he mixed oleo (beef tallow) and skimmed milk and added a strip of udder to mimic the way in which milk is curdled. He found that if he chilled, stirred and worked the mixture, it formed a white buttery mass with a pearly sheen, which he named after *margos*: Greek for pearl (Davidson 1999). This biological reasoning was completely wrong, but Mouriès had succeeded in producing a butter-like substance that has now become an indispensable staple on bread or as a cooking aid in large areas of the world.

The real microstructure and a schematic diagram of a typical margarine are shown together in Fig. 14.10. What is clear from the Cryo-TEM inset photo is that the margarine is inhomogeneous at a microscopic level, consisting of a finely divided water phase in a continuous phase comprising fat crystals and liquid oil. Conceptually we can think of margarine as a particle-filled gel in a plastic network as shown schematically beside the physical microstructure.

Margarine is technically an oil-in-water emulsion. Depending on legislation, full fat margarine has between 80% and 82% fat as this was the original benchmark definition of full fat butter which it had to mimic. As is well known, oil and water don't readily mix or stay mixed, but for full fat margarine it is almost impossible *not* to make a stable emulsion. This is due to the solid fat crystals present in the overwhelming continuous phase rapidly adsorb to the oil-water interface during the manufacture of the pre-emulsion and crystallise out upon cooling during processing: classical Pickering stabilisation.

The product has to be microbiologically safe, both in transit to the shops and afterwards during repeated use (open shelf-life). Additionally, it has to function as a heat transfer medium in the kitchen during cooking or baking, it functions as an ideal carrier of fat soluble flavours, and it improves the "mouthfeel" of bread by acting as a lubricant. It should also spread directly from the fridge without tearing the bread. It should be healthy by providing essential fatty acids, fat-soluble vitamins and aid in the uptake of other fat-soluble ingredients. Recent developments now offer cholesterol reduction with regular intake and margarine is an excellent vehicle to provide a delivery platform for functional ingredients in many parts of the world (e.g., Nestel et al. 2001).

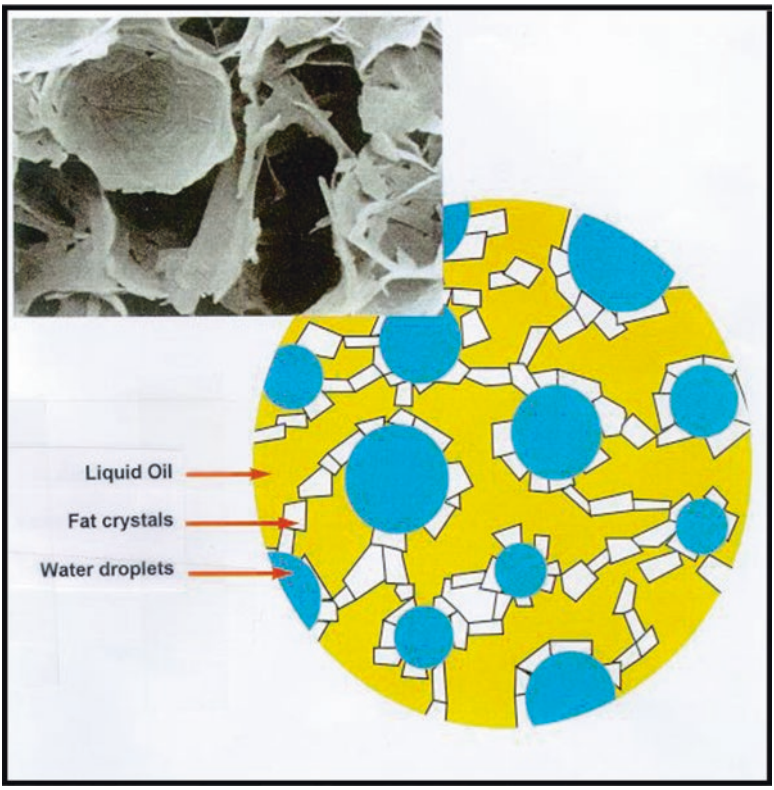

Fig. 14.10 Upper left corner: Cryo-SEM image of a fat crystal network in a 60% fat-continuous spread; oil and water have been removed from the sample for clarification. Reproduced with kind permission of Dr. I. Heertje, from Food Microstructure, Vol. 7 (1988), pp. 181–188

Margarine quality has come along way since the first crude products from 130 years ago. The first technological improvements were in the refinement of the triglyceride processing (hardening and fractionation) in the early twentieth century. This led to better tasting fat (less rancidity as metals were removed to reduce oxidation) and also allowed the "design tools" to manipulate melting curves for *blends* of fats to tailor margarine to different applications; e.g., frying, baking or spreading on bread (e.g., Bockisch 1993).

14.5.1 Low and Very Low-Fat Spreads

Around the mid 1970s, as consumers became more health conscious, the drive to lower fat levels in the edible fats sector began. Fat levels were reduced from the traditional 80% levels to 60% (reduced fat spread) and then through further development to 40% fat (low fat spread) in the 1980s. Processing of these so-called

reduced fat spreads was still the same as full fat, namely a fat-continuous process, but when the dispersed water phase volume reached 60% in the low-fat spreads, novel water phase control through process and emulsifiers was needed. Using the traditional process route at 40% fat resulted in water continuous systems so a new "inversion" process was developed. The choice of emulsifier was now crucial in controlling the balance between break-up and coalescence in the product to effectively force the equilibrium towards coalescence to drive phase inversion from a water continuous pre-mix to a fat continuous product.

This is a non-trivial challenge for the emulsion scientist. The product begins as a thin water continuous liquid pre-mix that is cooled under controlled shear until it phase inverts to become the thick spreadable plastic structure we know as margarine. However, if there is a problem during manufacture the cooled product has to be re-heated and re-worked back to a water continuous state where it is re-processed in the pre-mix tank. When the margarine is consumed it should also re-invert quickly in the mouth to provide salt release. Thus, there is a delicate interplay between small molecular weight monoglycerides and lecithins (fat continuous) and milk proteins (water continuous) to get the required emulsion stability during pre-mix, inversion, storage and in use.

The trend in fat reduction has continued where the technical limit based on conventional processing is around 20% fat. Holding 80% water in 20% fat is a challenge in collodial packing and can only be accomplished by using powerful water-in-oil promoting emulsifiers such as Admul wol (Polyglycerol polyricinolate). Effectively, the emulsion is beyond the close-packed limit for random spheres and as such exists as a polyhedral mass, where the internal pressure to re-coalesce and phase separate is high.

Fortunately, as little as 0.5% Admul Wol will emulsify and stabilise 80% water in 20% oil. Additional product stability can be gained by thickening the aqueous phase by biopolymers. However, the problem now shifts to making the spread destabilise in the mouth during mastication to allow salt release and to reduce unnecessarily thick mouthfeel. This is a difficult compromise to make; stable during processing, storage and spreading, becoming physically unstable during the transit time (mastication) in the mouth. A replacement for Admul Wol having the same physical stability but better mouthfeel is a Holy Grail in low or very low-fat spreads.

Another low-fat challenge is to obtain the same product functionality (cake baking, shallow frying, and on bread). For kitchen applications, fat is *the* perfect medium to transfer heat during the cooking process as it as a much higher boiling point than water. In particular for baking, the fat phase is crucial during the early steps of air incorporation and stabilising when whipping the cake batter. If the batter doesn't have enough entrained air, or the bubble size distribution is not optimal, then the final cake texture and crumb structure is not good. Solid (saturated) fat (SAFA) plays an important role here traditionally, so when one goes from 80% fat to say 40% fat the baking functionality is quickly lost. To restore this to the high fat standard required a change in emulsifier type and level. The supplementary use of alpha-tending emulsifiers (monoglyceride derivatives) or anionics (SSL, CSL) were found to compensate for the loss of SAFA upon reduction of fat level. In addition,

the use of mono-glycerides has a beneficial effect on the anti-staling of the starch allowing a longer shelf-life of the baked product (Wootton et al. 1967; Mizukoshi 1997).

At 60% fat, emulsifiers alone can compensate for reduced kitchen functionality, but when the fat level is reduced to 40%, processing necessitates that the water phase is thickened, typically by biopolymers such as starch or alginate. Then the kitchen performance is severely hampered as the biopolymers tend to burn or discolour during heating. In addition, spattering (explosive loss) of the water phase during frying becomes a bigger issue as the fat level is reduced. To combat spattering, lecithin is added to promote the flotation of water droplets to the air/oil interface during frying where they can harmlessly evaporate. Salt also has a positive effect on reducing spattering by functioning as anti-bump crystals during frying. In practice, the limit for general kitchen functionality is thus 60% fat where a viscous water phase is not needed for processing.

Duplex emulsions (O/W/O) have also been considered as a theoretically attractive route to lowering fat level as the internal water phase under some circumstances can "hide" some of the external fat phase. In practice, there are two main problems: processing is not perfect as the first emulsion W/O has to be carefully emulsified into the bulk fat/oil to make the O/W/O. Since emulsification requires shear, it is inevitable that the duplex emulsion is broken and thus yields are low. The second problem is coalescence of internal phase during storage, which again leads to loss of overall emulsion stability. Recent successes have been made with duplex emulsion manufacture using microfluidic devices. However, these currently manufacture at single drop rates so are many orders of magnitude too slow to be commercialised. Membrane emulsification has also shown promise in duplex manufacture, but with systems containing protein as one emulsifier, fouling and blocking of pores is a difficult problem here (Hitchon et al. 1999).

14.5.2 Zero-Fat Spreads (Lipogel Technology)

It is the ambition of many product developers to successfully replace all the fat in a product whilst maintaining acceptable sensory properties of the food product. In the case of spreads, which are generally a high fat food, this presents an incredibly challenging technical problem. A number of approaches have been tried including gelling bio-polymers, shear-gelled systems and the use of microparticulated proteins. One particular approach to this problem is to use the mesophase properties of monoglycerides and other emulsifiers in solution to design structures with acceptable material and sensory properties.

Figure 14.11 shows a typical phase diagram of a monoglyceride system. Given the relative simplicity of the chemistry of this system it is surprising how rich the microstructural possibilities are as witnesses by the different mesophase possibilities. A summary of this approach is that emulsifiers, due to their amphiphilic nature, display particular phase behaviour in solution in the absence of fat. For example,

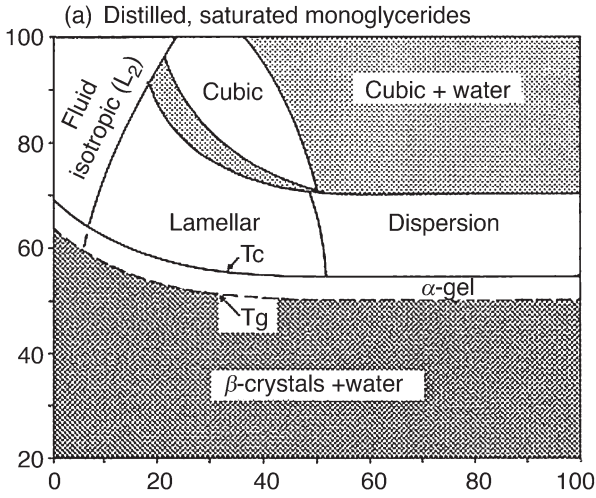

Fig. 14.11 Phase diagram showing possibilities of (edible) structured mesophases. (Taken from Dr. M. Zeelenberg Unilever Research Prize lecture Vlaardingen 1995, with permission)

through control of formulation and process, monoglyceride lamellar structures can be crystallised into water-swollen α-gel crystal structures which can provide spread-like textures, even for relatively low concentrations of emulsifier. Typical levels of emulsifier are 4–10%, which when processed properly, can hold 96–90% water in a plastic, spread-like rheology. These liquid crystal lamellar phases are sometimes called lipogels. Small amounts (5%) of fat can also be included but processing becomes critical.

Nutritionally, 1 g fat (SAFA) is comparable to around 20 g of lipogel, which offers a spreadable product at low nutritional load. This structuring/nutritional ratio would not be possible with conventional fat—continuous technology.

The main challenge of lipogel technology is to manipulate the phase behaviour of the emulsifier systems to provide the most appropriate crystalline structure for the particular application, thus optimising the rheological properties of the lipogel. Often co-emulsifiers are needed (depending on pH) however salt is known to interfere with mesophase formation. However, through appropriate use of emulsifier blending and processing it is possible to create lipogel structures for a wide range of applications, not just for use in zero-fat spread systems. In addition, because monoglygerides are lipid based, they can incorporate the same fat-soluble flavours and colorants normally used in high-fat margarine which is a distinct advantage above water-continuous products as zero-fat alternatives. Since lipogel contains high levels of surfactant, it performs surprisingly well as a baking margarine because the monoglycerides aid the aeration step during batter preparation. Other applications of the lipogel technology have now been extended to include zero fat dressings, mayonnaise, ice cream, whipping cream, and frozen desserts but the sensory properties of this class of products often differs from the high-fat version.

14.5.3 Oleogel Lipid Structuring

When used as part of product formulation, food lipids are present either as solids (fats) or liquids (oils). The material state of the lipid can be of significance to the overall material properties of the food, and thus the overall sensory experience. Whilst the presence of solid fat is neither necessary nor desirable in a product such as a salad dressing, it may conversely provide an essential structural contribution to products such as margarines, as well as butter, chocolate or cheese. As discussed earlier, the role of solid fat in each of these cases is primarily to provide physical stability and desirable material and textural properties to the product in question. The solid nature of edible fats is due to the presence of triglyceride fractions with melting points typically greater than ambient temperatures, and which provide crystallinity to the overall lipid structure. Melting point is determined by the chain length and degree of saturation of the fatty acid groups present within a triglyceride molecule, increasing in temperature with increasing chain length, but decreasing as the level of unsaturation is increased.

Pure fats have reasonably well-defined triglyceride compositions, and thus their melting and solid fat profiles are also well defined. For some products, such as butter or cheese, the use of a singular lipid source (i.e., butterfat) is sufficient to impart the appropriate material and organoleptic properties to the product. However, for other products, blending of different lipid fractions is necessary to achieve a solid fat profile appropriate to the technical and sensory properties of the product. For example, margarines and other non-dairy spreads formulated with polyunsaturated oils (that are invariably liquid under ambient conditions) require the addition of high melting point triglycerides based on saturated or *trans*-fatty acids in order to modulate the solid fat content towards a spreadable product. Conversely, more spreadable butters can be manufactured through appropriate blending or butterfat with mono or polyunsaturated oil.

While the presence of high melting point lipid fractions is an essential structural component of many semi-solid and solid fat-containing foods, a number of health issues have been associated with over-consumption of saturated and *trans* fats. In particular, the consumption of *trans* fats has been linked to reduced cardiovascular health, contributing to increased levels of low density lipoprotein (so-called bad cholesterol), whereas consumption of polyunsaturated fats has been attributed to the lowering of LDL cholesterol. Internationally, there have been significant legislative moves to eliminate the inclusion of *trans* fats from within food products, which has led to a need for the development of innovative technical solutions to replicate the structural contribution of *trans* fats (and to a lesser extent saturated fats) in soft solid and solid fat-based foods.

One particular technology that has garnered significant research and commercial interest as a means to provide structure and solidity to liquid oils, but without compromise to the nutritional profile of the lipid system, has been the use of oleogels, also termed organogels, lipid gels or oil gels. Research into this area has accelerated considerably over the past decade, and key findings have been well summarised in

a number of excellent, recent review articles, including those research works of Patel and Dewettinck in 2016 and by Co and Marangoni in 2012. Essentially, oleogels are liquid oil systems that contain an oil soluble or dispersible adjunct material that is able to undergo self-assembly within the oil to form a network of interconnecting or close-packed elements, thus restricting the mobility of the liquid oil and creating a gelled or solid-like state. Arguably, this is exactly what happens when saturated fats undergo crystallisation, causing entrapment of liquid oil (both interior and exterior to the crystal network) and resulting in solidification of the lipid state. However, the definition of oleogelation can be considered exclusive of the use of triglyceride fats *per se*, and can be distinguished in other ways, such as the ability to create structured, solid-like oil phases that are transparent in appearance and by the relatively low concentrations of adjunct required to cause gelation.

Based on the extensive research undertaken in this area in recent years, it has become evident that there is considerable diversity in the particular structuring pathways available for oleogelation, and in the materials available for achieving those pathways. The molecular diversity of oleogel structurants can range from small molecule hydrophobes and amphiphiles, through to large molecular weight biopolymers (Davidovich-Pinhas et al. 2016) and extending to the use of colloidal dispersions (noting that synergistic effects can often be achieved through the use of particular combinations). Oil structuring through the inclusion of biopolymeric materials requires the structuring component to have some affinity with the oil phase coupled with the ability to form percolating self-assemblies within the solvent to create a gelled state. This can be considered in the more familiar context of water structuring, in which the aqueous affinity of hydrocolloids such as polysaccharides is primarily driven through the preponderance of hydrophilic moieties (primarily hydroxyl) associated with the biopolymer chain that are compatible with the surrounding solvent phase. Gelation of polysaccharides in aqueous solution is then facilitated through various mechanisms of intermolecular interaction, usually through hydrogen bonding (e.g., agar) or electrovalent bridging (e.g., sodium alginate) leading to the formation of strong gels, or alternatively through molecular entanglement (e.g., xanthan) leading to the formation of weak gels. Biopolymer-mediated oil structuring therefore requires the presence of predominantly lipophilic groups across the polymer molecule that provide the requisite affinity with lipidic solvents, and some mechanism of self-association is required for gelation.

From a food manufacturing perspective, there are still only limited options for molecules fulfilling these requirements, with ethyl cellulose (additive number 462) one of the few food grade examples of a biopolymer able to form oleogels. Ethyl cellulose is produced through the alkalination of cellulose followed by addition of ethyl chloride, which reacts with the hydrophilic hydroxyl groups on the cellulose leading to their replacement with hydrophobic ethoxyl ether groups. The glucose units that comprise cellulose possess three hydroxyl groups available for substitution. Accordingly, and dependent on reaction conditions, the extent of this conversion can be controlled, thus altering the solution affinity of the molecule from essentially hydrophilic in the native state to amphiphilic at intermediate conversions through to increasingly lipophilic at high levels of ethoxyl ether substitution.

Commercial ethyl celluloses are typically produced with degrees of conversion ranging from 2.2 to 2.6 ethoxyl groups per glucose monosaccharide unit. Solubilisation of ethyl cellulose in oil requires conditions of high temperature (125–150 °C) and shear in order to overcome the glass transition point of the cellulose. On cooling, intermolecular association through the formation of hydrogen bond causes a sol-gel transition, producing optically transparent gels with soft, rubbery material properties. The degree of ethoxyl ether can have an impact on gel properties. High degrees of substitution may promote elongation of the polymer in solution, which should favour increasing gel firmness. However, highly substituted ECs may conversely possess fewer junction zones for gelation, as hydrogen bonding is favoured between hydroxyl groups. As well as variation in the extent of ethoxylation, the properties of ethyl cellulose can be altered through manipulation of the molecular weight of the polysaccharide backbone with gel firmness was seen to increase with increasing molecular weight of the EC (Davidovich-Pinhas et al. 2015).

Gel properties have also been found to be greatly influenced by oil type, even within similar classes of food oils. A study by Laredo et al. (2011) showed that gels produced using flaxseed oil and 10 wt% ethyl cellulose and were firmer than those produced by soybean oil, which in turn were firmer than those prepared with canola oil at equivalent concentrations of EC. Findings indicated that elastic modulus of the gel increased with the relative degree of unsaturation of the oil. The greater gel firmness observed with highly unsaturated oils was attributed to an increase in molar volume of the solvent, leading to greater polymer-polymer association by enhancing accessibility of junction zones.

The material properties of oleogels prepared with ethyl cellulose properties can also be influenced by the addition of surfactants (Dey et al. 2011). This is demonstrated in Fig. 14.12, which shows the effect of 5 wt% surfactant addition to flaxseed oil oleogels stabilised with 5 wt% ethyl cellulose. The addition of surfactant can act as a placticizer which can influence the gelation properties of the oleogel. As observed in Fig. 14.12, at equivalent concentrations, the specific type of added surfactant can have a profound effect on the material properties of the gel formed, not

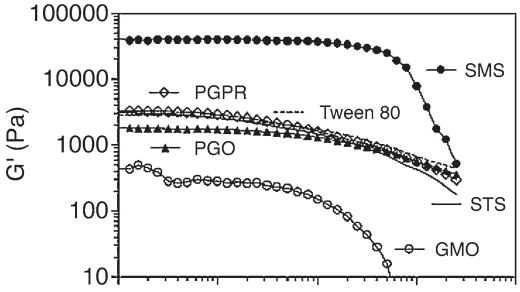

Fig. 14.12 Small deformation stress sweep of 90% flaxseed oil, 5 wt% ethyl cellulose gels containing 5 wt% surfactant (*SMS* sorbitan monostearate, *PGPR* polyglycerol polyricinoleate, *PGO* polyglycerol oleate, *STS* sorbitan tristearate, *GMO* glycerol monooleate). (Reproduced with permission from Dey et al. 2011)

only in regards to the relative elastic moduli at low applied stresses, but also in relation to the gel yield stress. Whilst the relationship between gel properties and emulsifier functionality, as defined by conventions such as hydrophilic-lipophilic balance, appears poorly defined, it was observed that the size of the surfactant head group did appear to correlate to the plasticizing effect, showing an increase in plasticization with decreasing head group size.

Oleogelation can also be achieved through a variety of lipid and polar lipid inclusions and mixtures thereof. Structuring by apolar lipids, such as waxes, requires heating of the oil and gelator such that the gelling component becomes molten within the solvent phase. Cooling of the solution leads to the crystallisation of the wax component through nucleation and association of neighbouring molecules through attractive forces, in particular van der Waals interactions, leading to growth of crystal structures. Percolation of these self-assembled crystalline structures provides the mechanical scaffolding to the lipid component, allowing gelation to take place. From a mechanistic perspective, wax crystallisation can be considered analogous to that of triglyceride crystallisation. However, differences in the molecular structure can lead to variations in packing arrangements, which can in turn influence macrostructural and material properties.

Wax oleogelation also differs from fat structuring using saturated triglycerides in that gel structures can be achieved at relatively low concentrations of added wax; for example, Toro-Vazquez et al. (2007) showed that Candelilla wax could be used to produce safflower oleogels at 2 wt% of added wax, whilst Dassanayake et al. (2009) demonstrated that oleogels could be formed with rice bran wax at concentrations as low as 0.5 wt%. The appearance and material properties of wax oleogels can also vary considerably depending on preparation variables such wax type, concentration and the influence of temperature (most notable cooling rate) and shear. In terms of appearance, whilst solid fats are invariably opaque, there can be considerable diversity in oleogel appearance, ranging from opaque to translucent to transparent, depending on the various length scales and dimensions of the self-assembled crystalline structures. Variations in optical clarity can equally impact on the relative colouration of the lipid system (Yilmaz and Ogutcu 2015), who compared the optical properties of oleogels prepared using either olive oil or hazelnut oil, and which were stabilised using beeswax or sunflower wax.

In terms of preparing gels from edible oils, a number of food grade waxes are available, including sunflower wax, rice bran wax, carnauba, and shellac (Dassanayake et al. 2012; Patel et al. 2013; Blake et al. 2014). Specific oleogelation characteristics are dependent on the molecular composition of these different waxes, with a general rule of thumb stipulating that waxes comprising molecules with longer hydrocarbon chains will be more effective gelators, on the basis that alignment of extended hydrocarbon chains results in greater structural reinforcement through greater van der Waals association. However, this hypothesis needs to be treated with some caution, since it might be argued that a similar correlation could be established between wax melting point and gelling properties, as melting point will equally be influenced by hydrocarbon chain length. Certainly, Dassanyake's study (Dassanayake et al. 2009), comparing the material properties of oleogels

prepared with 6 wt% carnauba, candelilla or rice bran wax, indicated that gel firmness did not scale with wax melting point, decreasing in the order rice bran (MP ≈ 76 °) > candelillia (MP ≈ 64 °C) > carnauba (MP ≈ 84). In this respect, gelling properties appear mostly determinant on the particular polymorphic nature and structure of the crystallites formed on cooling (Dassanayake et al. 2012). The influence of processing conditions can impact on the kinetics of crystallisation and subsequent structural assembly. Rate of cooling, in particular, was shown to be of consequence in regards to the structure of wax oleogels (Blake and Marangoni 2015).

14.6 Conclusions

The food industry has come a long way in the last few decades. Particularly now that most developed countries have the luxury of eating (often more than) enough calories per day the emphasis has shifted away from absolute level of fat or oil to *quality* of fat or oil. Thus, for example most spreads now sold typically contain 40% fat and less high fat margarine or zero fat spreads are sold today. In addition, the consumer is constantly looking for more functionality in the products. This can take many physical or nutritional forms such as easier spreadablilty or spoonability from the packaging, fortification (vitamins and minerals) and more recently to complex functional claims such as reduced cholesterol, blood pressure, improved satiety at reduced calories, or even improved mental performance (e.g., Upritchard et al. 2005).

For each of these functional claims there will typically be a preferred product format depending on functional active and even a preferred targeted part of the body to deliver the functional ingredient to. Manufacturers will have to use clever emulsion design rules to take account of break-down under physiological conditions to be able to make verifiable functional claims. For example, fast breakdown in the mouth boosts flavour release and salt perception; however, it may be necessary to get an intact emulsion into the stomach or even small intestine to claim proper (improved) bioavailability of a fortified product.

Such challenges between emulsion science, processing and nutritional demands will take functional food claims (and thus emulsion science) to a new level in the next decades in response to ever increasing consumer demand for healthy, nutritious and good tasting food.

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Chapter 15
Guidelines for Processing Emulsion-Based Foods

Ganesan Narsimhan, Zebin Wang, and Ning Xiang

15.1 Introduction

Emulsions are dispersions of one liquid into the second immiscible liquid in the form of fine droplets. Emulsions can be classified as either oil-in-water or water-in-oil emulsions depending on whether oil or water is the dispersed phase. Milk, cream and sauces are some examples of oil-in-water emulsions whereas butter and margarine are examples of water-in-oil emulsions. Ice cream and fabricated meat products are complex oil-in-water emulsions in which either additional solid particles are present or the continuous phase is semi-solid or a gel. Some examples of emulsions is shown in Table 15.1. Formation of emulsion results in a large interfacial area between two immiscible phases and therefore is usually associated with an increase in free energy. Consequently, emulsions are thermodynamically unstable, i.e. they will phase separate eventually. However, emulsifiers and proteins are usually employed in the formulation. They adsorb at the liquid-liquid interface thus lowering the interfacial tension. Smaller interfacial tension helps in the dispersion of one phase in the form of fine droplets by lowering the required interfacial energy.

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Table 15.1 Typical food colloids

Food	Type of emulsion	Method of preparation	Mechanism of stabilization
(1) Milk	O/W	Natural product	Protein membrane
(2) Cream	A + O/W	Centrifugation	As (1) + particle stabilization of air
(3) Ice cream	A + O/W	Homogenization	As (2) + ice cream network
(4) Butter and margarine	W/O	Churning and in votator	In crystal network
(5) Sauces	O/W	High-speed mixing and homogenization	By protein and polysaccharide
(6) Fabricated meat products	O/W	Low-speed mixing and chopping	Gelled protein matrix
(7) Bakery products	A + O/W	Mixing	Starch and protein network

Source: Darling and Birkett (1987)
O = oil; A = air; W = aqueous phase

In addition, the emulsifiers and proteins also modify the interdroplet forces thereby either preventing or retarding the rate of coalescence of colliding droplets during emulsion formation. Formulation therefore influences the size of emulsion drops formed using different types of emulsification equipment. Modification of interdroplet forces also helps in prolonging shelf life (kinetic stability) by slowing the rate of coarsening of emulsion drop size due to coalescence during storage. Proteins and emulsifiers also help in the extension of shelf life by providing rheological properties to the liquid-liquid interface. The main focus of this chapter is formation of emulsion. The chapter attempts to highlight the salient features of formation of emulsions and a brief description of different factors that control the drop size. Different types of emulsification equipment, the nature of flow field, breakup and coalescence of droplets and prediction of drop size during emulsion formation are discussed. No attempt has been made to discuss the mechanisms of destabilization of emulsion products during storage. Comprehensive treatments of this subject can be found elsewhere.

15.2 Emulsification Equipment

Many laboratory to large scale emulsion forming equipments are commercially available, Each type of equipment has its advantage and disadvantage. Selection of emulsification equipment depends on many factors, such as the scale of production, the properties of starting material, the desired drop size distribution, physicochemical properties of final emulsion, and capital and production costs. Main types of emulsification equipment are discussed below.

15.2.1 Colloid Mill

Colloid mill is a type of continuous emulsification equipment. Although there are many commercial designs available to obtain different performance, the principle of operation is quite similar. Figure 15.1 shows the schematic of a colloid mill. A gap was formed by the rotor and the stator, which is adjustable by the adjusting ring. Coarse emulsion is fed into the gap. High speed rotation of the rotor exerts high shear stress on the droplets and breaks them into smaller ones. The shear stress can be adjusted by changing the gap (usually from 50 to 1000 μm) and the rotation speed (usually from 1000 to 20,000 rpm) (McClements 1999). In addition to increasing shear stress, increasing residence time also decreases droplet size, either by decreasing the flow rate or recycling the products.

Many factors affect the operation of a colloid mill. High rotation speed, smaller gap thickness, low flow rate will make finer droplets albeit at higher energy consumption. Geometry and material of rotator/stator also affect the energy consumption and emulsion quality. Due to energy dissipation, the temperature of product will increase if no cooling system is associated with the mill. High temperature is unfavorable for the emulsion stability.

Colloid mill is suitable for processing intermediate to high viscosity fluids. Typical drop size from colloid mill is between 1 and 5 μm (McClements 1999). Usually the feed is pre-emulsion, because the efficiency of breakup droplets is much higher for pre-emulsions than for pure water and oil feeds.

15.2.2 High Speed Blender

High speed blender is a batch emulsification method most commonly used to disperse oil into liquid phase (Brennen et al. 1990). The liquid (oil and liquid phase) is placed into a vessel and agitated by a high speed stirrer. The product scale may be

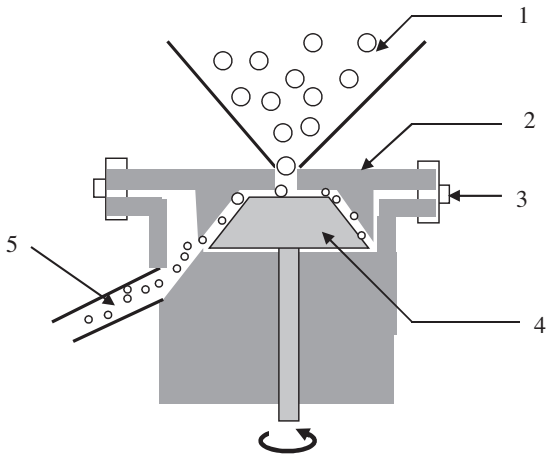

Fig. 15.1 Schematic of colloid mill. 1: Feed in (coarse emulsion); 2: stator; 3: adjusting ring; 4: rotator; 5: outlet (fine emulsion) (McClements 1999)

small (several cm^3 in a lab) to large (several m^3 in a plant). The stirrer rotates at rather high speed (usually hundreds of rpm to thousands of rpm) thus resulting in a velocity field with longitudinal, rotational and radial velocity gradients. Such gradients exert shear stress upon the fluid, disrupt the interface between the oil and the liquid phase, and finally form droplets. Because the velocity field highly depends on stirrer geometry, the efficiency of emulsion formation is strongly affected by the design of stirrer. There are a number of different types of stirrer available for different situations. Among them, the most commonly employed impeller is disk turbine. Turbine impellers create a predominantly radial flow field in the tank.

High speed blender is very useful for emulsions with low viscosity fluid. High rotational speed and longer stirring time result in a smaller droplet size. Typically, the droplet size obtained using a high speed blender is about 2–10 μm (McClements 1999).

During the stirring, energy dissipation also increases the temperature of emulsions. If long time stirring is needed, a cooling system is also necessary to control the temperature.

15.2.3 Pressure Homogenizer

The term homogenization means reduction of the droplet size of dispersed phase by forcing the coarse emulsion through a narrow channel at high velocity. Pressure homogenizer is a continuous equipment to produce fine emulsions. Like a colloid mill, it works at a much higher efficiency for pre-emulsions than for pure oil and liquid phase (Pandolfe 1995). Compared to colloid mill, it is more suitable for low and intermediate viscosity fluids.

The schematic of high pressure homogenizer is shown in Fig. 15.2 (Brennen et al. 1990). A valve and a valve seat form a narrow gap. Typical thickness of the gap is between 15 and 300 μm, which is adjustable in many commercial homogenizers. The pre-emulsion is pumped through the channel at high pressure. The pressure is adjusted by the adjusting handle in a pressure range, which depends on different designs. In some homogenizer, the pressure may high to 10,000 Psi. Once the coarse emulsions pass through the narrow gap, the pressure energy is converted to kinetic energy and the intense turbulent and shear stresses exerted on the droplet break them into small ones (Phipps 1985). Cavitation may also be responsible for the breakup of emulsion droplets (Phipps 1985).

Smaller gap thickness and/or higher homogenizer pressure will result in smaller droplet size. However, this will require more energy. Because the residence time in the homogenizer is usually very small, it is possible that the emulsifying agent is poorly distributed over the newly created liquid-liquid interface, especially when the emulsifying agent is protein. In such cases, the fine droplets that leave the homogenizer tend to cluster and clump. To overcome this, a “two-stage” homogenization process is applied in some commercial homogenizers (Brennen et al. 1990). The coarse emulsions pass through a high pressure stage to break up the droplets, and then enter a low pressure valve to disrupt any clumps that may have been formed.

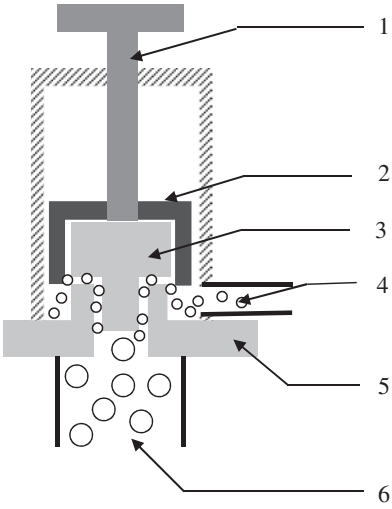

Fig. 15.2 Schematic of pressure homogenizer. 1: Pressure adjusting handle; 2: breaker ring; 3: valve; 4: outlet (fine emulsion); 5: valve seat; 6: inlet (coarse emulsion) (Brennen et al. 1990)

High pressure homogenizer is very efficient to reduce the droplet size of an emulsion. Typical droplet size is about 0.1 μm, and in some homogenizers it may be as low as 0.02 μm (Brennen et al. 1990). Another advantage is that the temperature increase is small unless the emulsions are recycled several times in multiple passes or the homogenizer pressure is extremely high.

15.2.4 Other Equipment

Besides the three types of emulsion forming equipment discussed above, there are several other types of equipment available to produce emulsions. Because they are not used as extensively as the above ones, they are discussed briefly below.

Ultrasonic homogenizers utilize high-intensity ultrasonic waves to generate intense shear stress and pressure gradient (Gopal 1968; McCarthy 1964). Such stress and gradient are responsible for disruption of the droplets. Two types of methods are used to generate ultrasonic waves: piezoelectric transducers and liquid jet generators (Gopal 1968). Piezoelectric transducers are ideal for preparing small volume of emulsions. It is a batch operation suitable for a laboratory use. The minimum droplet size may be as low as 0.1 μm. Liquid jet generator can be operated continuously. Compared to high pressure homogenizer, the energy efficiency is better, while the minimum droplet size is about 1 μm (Brennen et al. 1990).

Microfluidization is used to form emulsions with extremely small droplet size (may be smaller than 0.1 μm) (Dickinson and Stainsby 1988). In microfluidization, the two phases are separately accelerated to a high velocity and then simultaneously hit on a surface. As a result, the dispersed phase is broken into small droplets. By recycling the emulsion, the droplet size may be reduced.

Membrane homogenizers use glass membranes with uniform pore size to create droplets (Kandori 1995; McCarthy 1964). The dispersed phase is forced into the continuous phase. Because the droplet size strongly depends on the pore size of membrane in addition to interfacial tension between the dispersed and continuous phases, droplet size distribution of the product is very narrow. Also, the droplet size can be controlled by the membrane pore size. Another advantage is that the energy efficiency is high because of less energy dissipation compared to other emulsification equipment. The droplet size can be controlled to vary in the range of 0.3 and 10 μm.

15.3 Droplet Phenomena

As a colloidal system, emulsion consists of large amount of small droplets. Droplet size and droplet size distribution has remarkable effects on the stability and texture of final product (Dickinson 1992; McClements 1999). The goal of emulsification is to form fine droplets, which depends on the breakup (or, technically, *disrupture*) of large droplets in to smaller droplets. Due to the thermodynamical instability of colloidal system, small droplets tend to merge into larger ones, which is called *coalescence*. Drop breakup and coalescence are two contrary processes exist in emulsification, as shown in Fig. 15.3. High shear stress distort and breakup large droplets into small ones. If the emulsifier reagents adsorbed fast enough to stabilize the small droplets, they will be exist as small droplets. Otherwise, they will collide and emerge into larger droplets. The droplet size distribution of an emulsion product in an emulsification equipment depends on the balance between the two processes. The mechanism of breakup and coalescence will be discussed below.

15.3.1 Drop Breakup

In order to form an emulsion, one phase is to be broken up in the form of fine droplets and dispersed in the second continuous phase. The interfacial energy of the emulsion is proportional to the interfacial area of the emulsion droplets and the

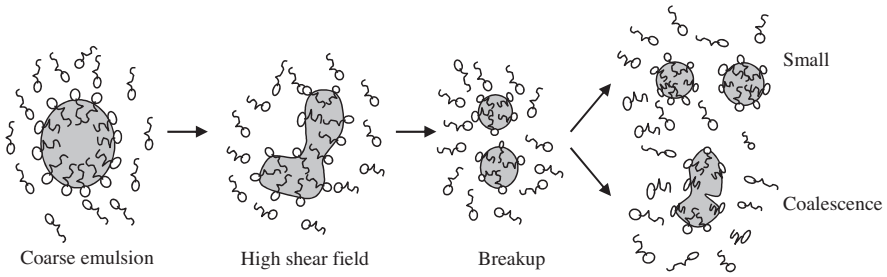

Fig. 15.3 Droplet breakup and coalescence in a high shear field

interfacial tension. Breakup of one phase in the form of fine droplets would result in an increase in the interfacial area and therefore would require an increase in the interfacial energy. Consequently, external energy input is necessary to increase the interfacial area. In order to minimize the interfacial energy, an emulsion droplet will assume spherical shape so as to minimize the surface area per unit volume. The surface energy of the droplet E is given by,

$$E = 4\pi\gamma R^2 \tag{15.1}$$

where γ is the interfacial tension and R is the droplet radius. Because of the radius of curvature of the droplet there is an interfacial stress or Laplace pressure p_l acting on the droplet which is given by,

$$p_l = \gamma\left(\frac{1}{R_1} + \frac{1}{R_2}\right) \tag{15.2}$$

R_1 and R_2 being the principal radii of curvature of the interface. For a spherical droplet, the above equation reduces to,

$$p_l = \frac{2\gamma}{R} \tag{15.3}$$

Any deformation of the droplet for its breakup will result in a decrease in its principal radii of curvature and therefore will require an increase in the interfacial stress acting on the drop surface. This increase in the interfacial stress is provided by an external flow that is induced in the continuous phase through energy input. It can easily be visualized that this external interfacial stress that is necessary for drop breakup is higher for smaller droplet. Since the droplet size is in the range of 0.1 to a few micrometers in food emulsion,[1] extremely high energy input is usually necessary to break up coarse emulsion into such small sizes. Typical energy input for emulsion formation can range from 10^7 to 10^{12} W/m^3. It should also be noted that only a small fraction of energy goes into the increase in the interfacial energy due to an increase in surface area. Most of the energy input is dissipated in the form of heat. Of course, this interfacial stress (and therefore the interfacial energy) can be decreased by decreasing the interfacial tension by the addition of food emulsifiers.

In a Colloid mill, the two phase mixture is subjected to extremely high shear when the mixture is passed through a narrow gap between a stator and a rotor. When the mixture is passed through a sudden contraction, such as a small orifice or pores of a membrane, the two phase mixture is subjected to hyperbolic/extensional flow. There may be other instances where a combination of these two types of flows may be encountered. These types of flow fields are laminar, i.e. the droplet Reynolds number (which is a measure of inertial and viscous forces) is very small.

[1] The droplet size needs to be as small as possible in order to reduce the rate of creaming as well as Brownian collisions so as to minimize coarsening due to coalescence.

Consequently, the external stress that is applied to counteract the interfacial stress is predominantly viscous. Extensive investigations of drop breakup due to laminar flow have been carried out. A brief overview of these results will be given here.

15.3.1.1 Laminar Flow

Experimental observations of drop deformation in hyperbolic (Rumscheidt and Mason 1961a) and simple shear (Karam and Bellinger 1968; Taylor 1932, 1934; Torza et al. 1972) flows indicated that the drops deformed in the form of prolate ellipsoid for low deformations. The hyperbolic flow was generated by a four-roller apparatus (Rumscheidt and Mason 1961b) whereas the shear flow was generated by a couette device (Bartok and Mason 1957). These experiments were conducted for a range of viscosity ratios varying from 1.3×10^{-4} to 29. Taylor (1932) observed that the mode of burst of the droplets depended on the type of flow and viscosity ratio. The flow fields for these flows are given by,

$$\text{Hyperbolic flow}: \quad u' = \frac{Gx'}{2} \quad v' = \frac{Gy'}{2} \quad w' = 0 \tag{15.4}$$

$$\text{Shear flow}: \quad u = Gy \quad v = 0 \quad w = 0 \tag{15.5}$$

where u, v, w are the velocity components along axes X, Y, Z respectively and u', v', w' are velocity components along axes X', Y', Z' respectively as shown in Fig. 15.4. G is the shear rate. Hyperbolic flow is irrotational whereas shear flow is rotational with a rotation of $G/2$. A drop suspended in the middle of four-roller apparatus was distorted into a prolate ellipsoid oriented along X', the deformation increasing with shear rate G. This continues until a critical shear rate G_B above which the drop bursts. For low viscosity ratio (drops of viscosity much smaller than the continuous phase), the deformed ellipsoidal drop developed pointed ends beyond the critical shear rate G_B eventually breaking off at the ends to form small satellite drops. For higher viscosity ratios, however, instead of developing pointed ends, the drop deformed into a thread which broke to form several daughter droplets.

For shear flow, at low G, the principal axis of prolate spheroid was $\pi/4$. The deformation and the angle of rotation both increased with G. Detailed analysis of four different types of deformations for different viscosity ratios is described (Rumscheidt and Mason 1961a) and shown in Fig. 15.5. At low viscosity ratio, the drop assumed a sigmoidal shape with the angle greater than $\pi/4$, pointed ends were formed from which fragments were released. At intermediate viscosity ratios, when the shear rate reached the critical value G_B, the central portion of the drop suddenly extended into a cylinder which formed a neck eventually breaking into two identical daughter droplets and three satellite fragments. At even higher viscosity ratio, the drop extended into a thread which increased in length until it broke into a large number of daughter droplets. At very high viscosity ratio, the drop deformed into an ellipsoid with the angle of rotation reaching $\pi/2$. No drop breakup was observed.

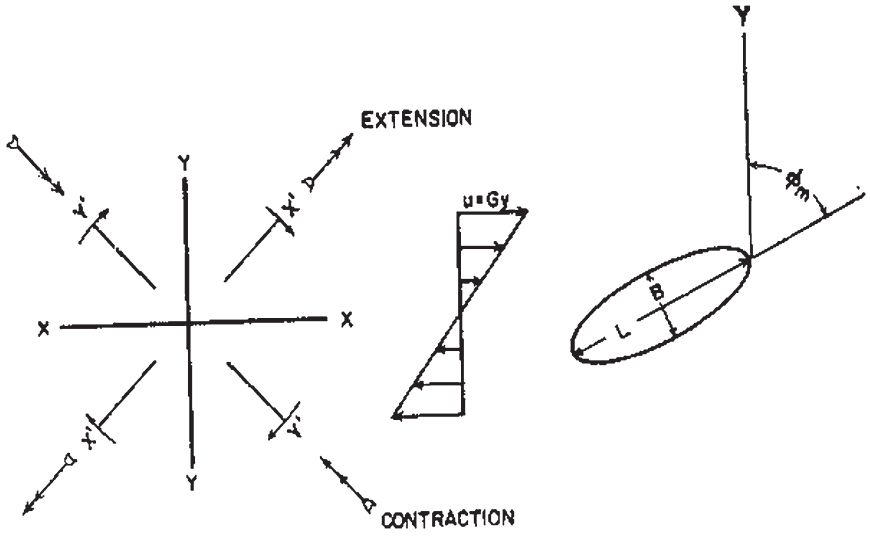

Fig. 15.4 Coordinate systems for hyperbolic and shear flow. The two fields are equivalent when X′Y′ axes are 45° behind XY axes and rotated clockwise at a rate G/2 as indicated on the left. The principal deformation axes are indicated by the double arrows. The parameters of a deformed fluid drop are shown on the right (Rumscheidt and Mason 1961a)

General analysis of drop deformation in an external flow involves solution of the velocity field outside in the vicinity of the drop as well as inside the droplet. For creeping flow (low Reynolds numbers) the equations of continuity and motion in the vicinity of the drop are given by (Barthes-Biesel and Acrivos 1973; Cox 1969; Torza et al. 1972),

$$\nabla.\mathbf{U} = 0 \tag{15.6}$$

$$\nabla^2\mathbf{U} - \nabla P = 0 \tag{15.7}$$

where $\mathbf{U}$ and P are the dimensionless velocity and pressure respectively, all the quantities being non-dimensionalized with respect to characteristic velocity, continuous phase viscosity and drop size. Similar equations can be written for fluid flow inside the drop. The solution of the above equations for velocity and pressure can be written in terms of spherical harmonics. The flow field $\mathbf{U}$ and the flow field $\mathbf{U}^*$ inside the drop can be as expansion in terms of a small perturbation parameter ε as (Barthes-Biesel and Acrivos 1973)

$$\mathbf{U} = \mathbf{U}_0 + \varepsilon\mathbf{U}_1 + \varepsilon^2\mathbf{U}_2 \tag{15.8}$$

$$\mathbf{U}^* = \mathbf{U}_0^* + \varepsilon\mathbf{U}_1^* + \varepsilon^2\mathbf{U}_2^* \tag{15.9}$$

In the above equation, $\mathbf{U}_0$ and $\mathbf{U}_0^*$ are the continuous phase and drop phase velocity fields for undeformed spherical drop shape respectively, $\mathbf{U}_1$, $\mathbf{U}_2$, $\mathbf{U}_1^*$ and $\mathbf{U}_2^*$ are the

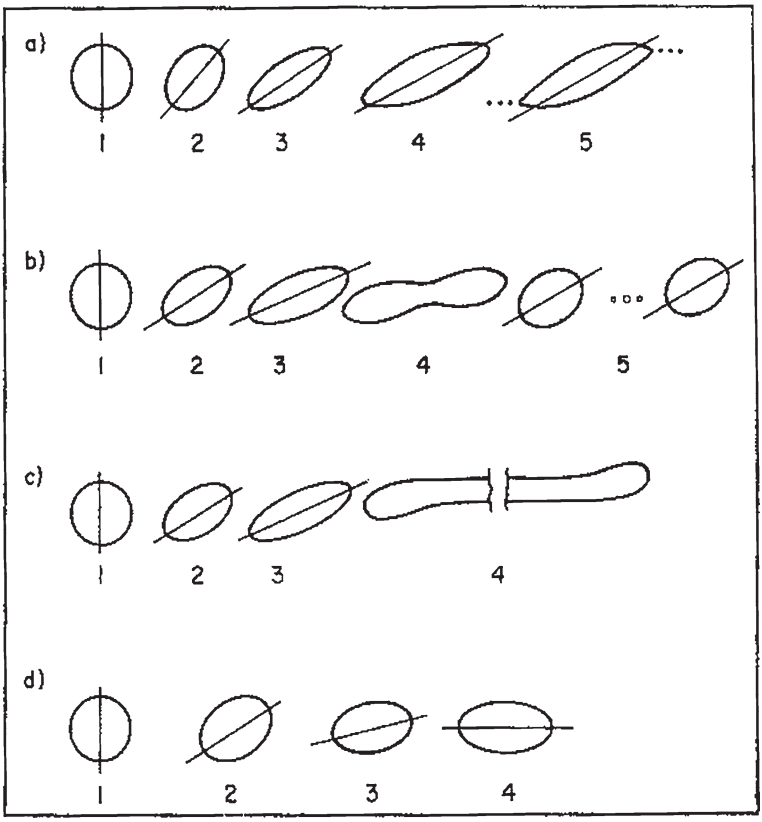

Fig. 15.5 Tracings from photographs of drops in shear flow showing the change in D, φ_m with increasing G up to breakup. (**a**) Class A deformation. (**b**) Class B deformation, the neck formed disintegrated into three satellite droplets. (**c**) Class B-2 deformation, drops were drawn out into long cylindrical threads. (**d**) Class C deformation, no burst was observed (Rumscheidt and Mason 1961a)

first and second order deviations. Since the creeping flow equations are linear, the velocity fields for undeformed drop shape as well as deviations satisfy the creeping flow equations. The shape of the deformed drop surface can be written as (Barthes-Biesel and Acrivos 1973),

$$r = 1 + \varepsilon f_1\left(r_1/r, r_2/r, r_3/r\right) + \varepsilon^2 f_2\left(r_1/r, r_2/r, r_3/r\right) \tag{15.10}$$

where f_1 and f_2 are shape functions. These equations have to be solved to obtain the shape functions recursively with the boundary conditions (1) normal components of velocity at the interface are continuous, (2) the tangential components of velocity at the interface are continuous, (3) the tangential components of the stress at the interface are continuous and (4) the difference in the normal components of the stress at the interface is counterbalanced by the interfacial tension forces due to curvature of

the interface. The curvatures of the interface are expressed in terms of shape functions.

The deformation of the drop depends on two dimensionless parameters, namely, $\lambda = \mu^*/\mu$ and $k = \gamma/\mu Gb$. Taylor's (1932) theoretical analysis of drop deformation in a shear flow considered the case for which the interfacial tension effects are dominant over viscous effects, i.e. $\lambda = O(1)$; $k \gg 1$ and obtained drop deformation to order k^{-1}. The drop was shown to deform into a spheroid with its major axis at an angle of $\pi/4$. He also considered the case for which the interfacial tension effects were negligible, i.e. $k = O(1)$; $\lambda \gg 1$ in which the drop deformation was obtained of order λ^{-1}. In this case, the drop deformed into a spheroid with its major axis in the direction of flow. This was then extended to the general case of drop deformation expressed in terms of a small deformation parameter upto a first order (Cox 1969) and second order (Barthes-Biesel and Acrivos 1973). The drop deformation was expressed in terms of a deformation parameter D defined as,

$$D = \frac{(L-B)}{(L+B)} \tag{15.11}$$

L and B being the lengths of major and minor axes respectively of the deformed drop. For steady shear flow, the drop deformation and orientation are given by (Torza et al. 1972),

$$D = \frac{1}{k}\left(\frac{19\lambda+16}{16\lambda+16}\right) \tag{15.12}$$

$$\alpha = \frac{\pi}{4} + \frac{1}{k}\frac{(19\lambda+16)(2\lambda+3)}{80(1+\lambda)} \tag{15.13}$$

When the droplet is exposed to a sudden shear flow, the transients of deformation of the droplet has been solved upto first order by Torza et al. (1972)

$$D' = D\left[1-2e^{-20Gkt/19\lambda}\cos(Gt)+e^{-40Gkt/19\lambda}\right]^{1/2} \tag{15.14}$$

where the steady state deformation D is given by,

$$D = \frac{5(19\lambda+16)}{4(1+\lambda)\sqrt{(19\lambda)^2+(20k)^2}} \tag{15.15}$$

The drop undergoes a transient damped wobble with a relaxation time $\tau = b\mu^*/\gamma$. Experimental observation of steady and transient deformations agree well with theoretical predictions for shear and hyperbolic flows.

The effect of external force on drop deformation can be described by a dimensionless number *We*, Weber number defined as,

$$We = \frac{\mu G b}{2\gamma} \tag{15.16}$$

which is the ratio of applied shear stress and Laplace pressure. The drop deformation increases with Weber number eventually resulting in drop breakup at a critical Weber number We_{cr}. The maximum stable drop diameter $d_{\max}$ is given by,

$$d_{\max} = \frac{2\gamma We_{crit}}{\mu G} \tag{15.17}$$

Theoretical prediction of drop breakup at a given imposed shear rate (Weber number) was accomplished from the minimum deformation parameter at which no solution exists for drop shape (Barthes-Biesel and Acrivos 1973). Experimental observation of critical Weber number for different types of flows for different viscosity ratios is shown in Fig. 15.6. The figure also shows the slope $d \log We_{cr}/d \log \lambda$ as a function of viscosity ratio λ. This can be used to predict the effect of change in viscosity ratio on drop breakup. For low viscosity ratios, We_{cr} decreased with λ for simple shear and plane hyperbolic flows and reached a minimum. For simple shear flows, We_{cr} increased dramatically at higher viscosity ratios and reached infinity at a critical λ of around 4 indicating thereby that highly viscous drops do not rupture when exposed to simple shear. Such a behavior was not encountered for other flows. Also, We_{cr} was found to be much smaller for plane hyperbolic and extensional flows implying thereby that it is easier to break a drop in these flow fields.

Experimental measurements of drop size of an emulsion produced in a colloid mill for excess surfactant concentration at different dispersed phase viscosities were employed to infer We_{crit} (Walstra and Smulders 1998). The results are shown in Fig. 15.7. The results seem to agree reasonably well with the values for single drops in simple shear flows for viscosity ratios up to about 2. Interestingly, drop breakup was observed for viscosity ratio as high as 10 rather than 4 as predicted for single drops which the authors attribute to possible contribution of elongation to the flow field.

15.3.1.2 Turbulent Flow

In a high pressure homogenizer, the two phase oil-water mixture is forced through a small gap between two plates at a high pressure. The pressure energy is converted to kinetic energy when the fluid flows through the small opening. In addition, the fluid is also subjected to high shear. Because of the large velocity of the fluid through the opening, the flow is highly turbulent, i.e. the Reynolds number is very large. The mechanism of drop breakage under these conditions is different from that in a laminar flow field. Because of turbulence, the droplet surface is subjected to random velocity and pressure fluctuations thus resulting in a bulgy deformation of the drop surface. In order for the droplet to rupture, the turbulent stresses acting on the drop surface should overcome the restoring force due to interfacial tension. The turbulent stress τ_{tur} is given by (Hinze 1955),

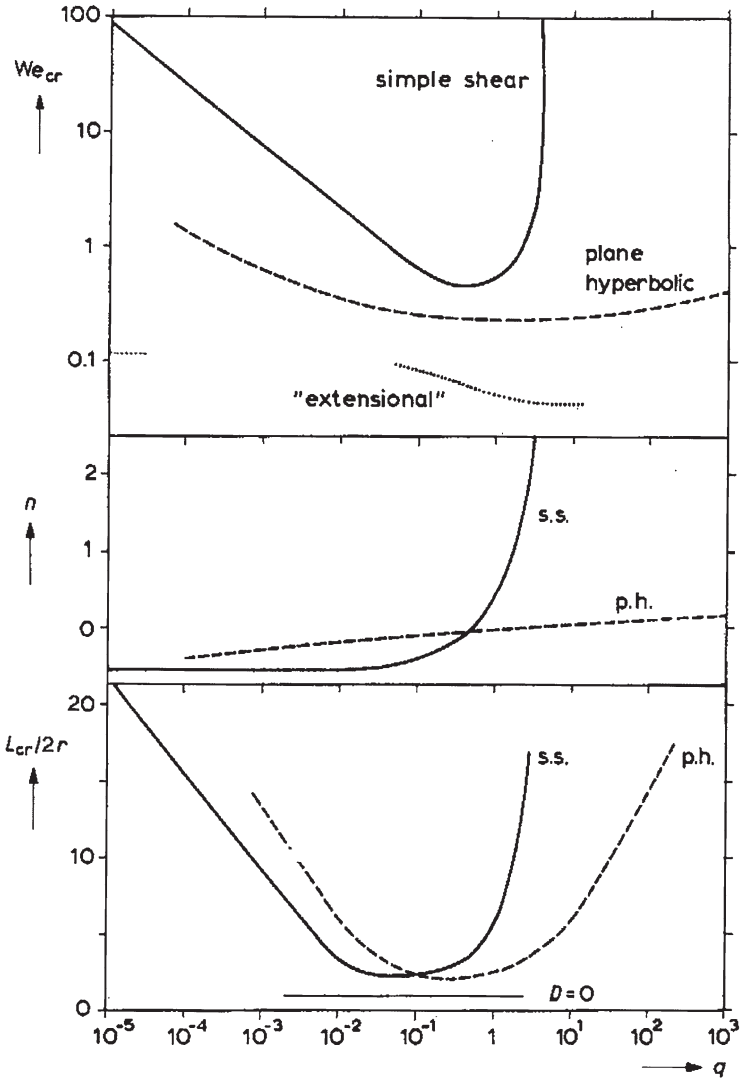

Fig. 15.6 Critical Weber number for breakup We_{cr} (i.e., the drop will break in the region above the curve); viscosity exponent $n = d \log We_{cr}/d \log \lambda$; and the largest drop dimension at burst L_{cr} relative to original drop diameter; for various types of steady flow as a function of viscosity ratio q (Walstra 1983)

$$\tau_{tur} = \rho \overline{u^2(d)} \tag{15.18}$$

where ρ is the density of the continuous phase and $\overline{u^2}$ is the mean square of the relative velocity fluctuations between two diametrically opposite points on the drop surface. Similar to the laminar case, one can define the dimensionless Weber number

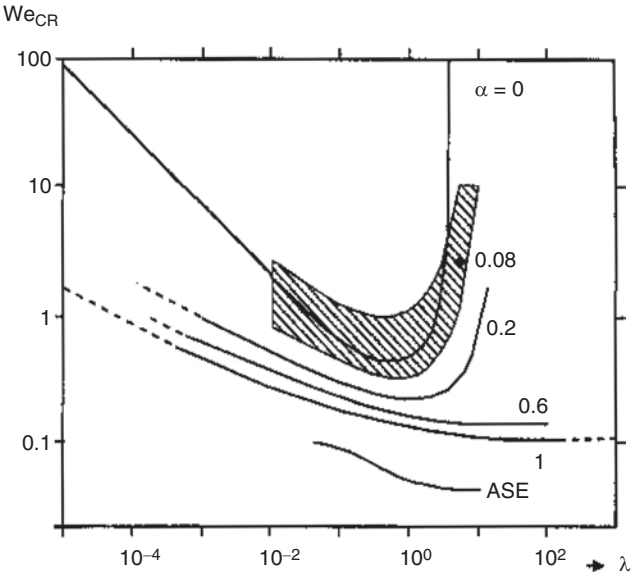

Fig. 15.7 Critical Weber number for break-up of drops in various types of flow. Single-drop experiments in two-dimensional simple shear (α = 0), hyperbolic flow (α = 1) and intermediate types, as well as a theoretical result for axisymmetrical extensional flow (ASE). The hatched area refers to apparent We_{cr} values obtained in a colloid mill (Walstra and Smulders 1998)

$$We = \frac{\tau_{tur} b}{2\gamma} \tag{15.19}$$

as the ratio of turbulent stress and Laplace pressure. Experimental observations (Hinze 1955) have indicated that the critical Weber number We_{cr} at which drop breakup occurs is close to unity. Consequently, the maximum stable drop diameter $d_{\max}$ is given by,

$$d_{\max} \simeq \frac{4\gamma}{\tau_{tur}} = \frac{4\gamma}{\rho \overline{u^2\left(d_{\max}\right)}} \tag{15.20}$$

The velocity fluctuation in a turbulent flow field can be considered as superposition of disturbances of different wavenumbers (wavelengths). Each wavenumber corresponds to a correlation lengthscale of disturbance. Turbulence that is generated is of lengthscale corresponding to the lengthscale of equipment. The turbulence energy is then transmitted to smaller lengthscales. Since the viscous forces become important for sufficiently small lengthscales (large wavenumbers), this energy is eventually dissipated over those length scales in the form of heat. Eventhough the turbulent flow field is anisotropic over large lengthscales, for sufficiently large Reynolds numbers, the flow field is locally isotropic over lengthscales comparable to drop sizes encountered in food emulsions. The unique feature of local isotropy is that the

flow field over these lengthscales do not depend on the characteristics of turbulent forming equipment. The energy spectrum over these lengthscales is universal in that it depends only on energy dissipation rate per unit mass ε, density ρ and viscosity μ. The universal spectrum can further be subdivided into lengthscales of inertial and viscous subrange. In the inertial subrange, the energy is just convected from larger to smaller eddies whereas in viscous subrange part of the energy is also dissipated. The demarcation between the two subranges is the microscale of turbulence λ_m given by,

$$\lambda_m = \mu^{3/4} \rho^{-3/4} \varepsilon^{-1/4} \tag{15.21}$$

Eddies of size greater than λ_m mainly convect energy without dissipation and viscous dissipation is important only for eddies of size smaller than λ_m. From dimensional analysis, it has been shown by Kolmogorov (Levich 1962) that the mean square velocity fluctuation over lengthscale in the inertial and viscous subranges are given by,

$$\overline{u^2(l)} \approx \varepsilon^{2/3} l^{2/3} \quad l(>\lambda_m) \tag{15.22}$$

$$\overline{u^2(l)} \approx \varepsilon \rho l^2 \mu^{-1} \quad l(<\lambda_m) \tag{15.23}$$

Using the above equation in the expression for the turbulent stress acting on a drop and from Eq. (15.18), the maximum stable diameter $d_{\max}$ in the inertial subrange is given by,

$$d_{\max} \approx \varepsilon^{-2/5} \gamma^{3/5} \rho^{-1/5} \tag{15.24}$$

Of course the above expression is applicable only if $d_{\max} > \lambda_m$. Similarly, the maximum stable diameter $d_{\max}$ in the viscous subrange is given by,

$$d_{\max} \approx \gamma^{1/3} \varepsilon^{-1/3} \left(\frac{\mu}{\rho} \right)^{1/3} \tag{15.25}$$

Of course the above expression is applicable only if $d_{\max} < \lambda_m$.

An analysis of drop breakup accounting for dispersed phase viscosity has been proposed (Calabrese et al. 1986). The disruptive turbulent stress acting on the droplet is assumed to be counterbalanced by cohesive interfacial tension force and viscous stresses that are generated inside the droplet for a maximum stable drop size. The following correlation was derived for droplets in the inertial subrange,

$$\frac{\rho_c \varepsilon^{2/3} d_{\max}^{5/3}}{\sigma} = C_5 \left[1 + C_6 \left(\frac{\rho_c}{\rho_d} \right)^{1/2} \frac{\mu_d \varepsilon^{1/3} d_{\max}^{1/3}}{\sigma} \right] \tag{15.26}$$

where C_5 and C_6 are constants.

In a high pressure homogenizer, the pressure energy is converted to turbulent kinetic energy. Since the kinetic energy is eventually dissipated in the form of heat due to viscous dissipation, the energy dissipation per unit mass can be written as inlet homogenizer gauge pressure P_h (which is pressure drop through the homogenizer since the outlet pressure is atmospheric). Therefore, the energy dissipation rate per unit mass ε can be written as,

$$\varepsilon = \frac{P_h}{\theta} \tag{15.27}$$

where θ is the residence time of the fluid through the homogenizer valve. Using Bernoulis equation, the average velocity through the homogenizer $\bar{U}$ can be approximated as $\bar{U} = \left(P_h / \rho\right)^{1/2}$. Recognizing that the residence time $\theta = Z / \bar{U}$, Z being the path length of the homogenizer, we have,

$$\varepsilon = P_h^{3/2} \rho^{-1/2} Z^{-1} \tag{15.28}$$

Breakup of a droplet also depends on the time the disrupting force acts on the droplet. If the turbulent force does not act for sufficiently long time, the droplet will not be disrupted effectively. In order for the drop to deform, the eddy time (time during which the force fluctuation acts) should be larger than the drop deformation time. The eddy time $\tau(d_{\max})$ for maximum stable drop size is given by,

$$\tau\left(d_{\max}\right) = \frac{d_{\max}}{\left(\overline{u^2\left(d_{\max}\right)}\right)^{1/2}} \tag{15.29}$$

where $\overline{u^2\left(d_{\max}\right)}$ is given by either Eq. (15.22) or (15.23) depending on whether the drop size is in inertial or viscous subrange. The drop deformation time $\tau_{def}(d_{\max})$ is given by,

$$\tau_{def}\left(d_{\max}\right) = \frac{\mu_d d_{\max}}{2\gamma} \tag{15.30}$$

Therefore, it is more difficult to breakup droplets in same eddy time with higher dispersed phase viscosity. Some sample calculation of droplet disruption is shown in Table 15.2 (Walstra 1983). It is seen that deformation times are usually smaller than eddy times, unless the ε or μ_d is extremely high.

15.3.1.3 Drop Breakage Rate

In addition to the characterization of maximum stable drop size, a number of mechanisms for drop breakage in a turbulent flow have been proposed. These models consider the deformation of a drop due to interaction with the turbulent flow field and the probability of breakup of a deformed droplet (Coulaloglou and Tavlarides

Table 15.2 Sample calculations for droplet disruption in isotropic turbulent flow (only order of magnitude is shown) (Walstra 1983)

Variable[a]	Unit	Tank with stir	Ultra turrax	Homogenizer
ε	W/m^3	10^4	10^{18}	10^{12}
l_e	μm	18	1.8	0.2
d_{max}	μm	400	10	(0.25)[b]
d_{min}	μm	0.3	0.3	0.3
$\tau(d_{max})$	μs	2500	10	(0.04)[b]
$\tau_{def}(\mu_d = 10^{-3})$[c]	μs	20	0.5	0.01
$\tau_{def}(\mu_d = 1)$[c]	μs	2×10^4	500	10
$d_{max}(if \ll l_e)$	μm	(3000)[b]	30	0.3

[a]Other variables: $\gamma = 10$ mN/m, $\rho_c = 10^3$ kg/m^3, $\mu_c = 10^{-3}$ Pa · s
[b]Theory does not hold here
[c]For a globule of size d_{max}

1977; Narsimhan et al. 1979). The rate of breakage $\Gamma(d)$ of a droplet of diameter d is written as,

$$\Gamma(d) = \text{Eddy drop collision frequency} \times \text{breakage efficiency} \tag{15.31}$$

In one model, the eddy arrival rate on a drop surface is visualized as a Poisson process. The relative velocity fluctuation between two diametrically opposite points of a droplet is assumed to be a normal distribution. Based on the assumption that the energy required for drop breakup is the increase in the surface energy of daughter droplets for binary equal breakup, the following expression for drop breakage rate was derived (Narsimhan et al. 1979):

$$\Gamma(d) = \lambda erfc\left(\frac{a\sigma^{1/2}d^{-5/6}}{\rho^{1/2}\varepsilon^{-1/3}}\right) \tag{15.32}$$

where λ is the rate of arrival of eddies, a is a constant and σ is the interfacial tension. In the other model (Coulaloglou and Tavlarides 1977), the rate of collisions of eddies with the droplet was calculated from the knowledge of the energy spectrum and the probability of drop breakup was assume to be exponential. The following expression was derived for the rate of breakage

$$\Gamma(d) = k_1 \frac{\varepsilon^{1/3}}{(1+\phi)d^{2/3}} \exp\left[-k_2 \frac{\sigma(1+\phi)^2}{\rho_d \varepsilon^{2/3} d^{5/3}}\right] \tag{15.33}$$

where k_1 and k_2 are constants, ϕ is the dispersed phase fraction and ρ_d is the density of dispersed phase.

The drop breakage rates have been inferred from the experimental data of transients of drop size distributions in stirred lean liquid-liquid dispersions (Narsimhan et al. 1980, 1984; Sathyagal et al. 1996) using similarity analysis. The inferred breakage functions were non-dimensionalized with respect to the natural frequency of oscillation of drops to yield a satisfactory generalized plot against dimensionless drop volume. Experimental data were correlated to give the following equation (Narsimhan et al. 1980, 1984)

$$\Gamma(v)\sqrt{\frac{\rho v}{\sigma}} = 5.75We^{3.2}\left(\frac{v}{D^3}\right)^{1.78} \tag{15.34}$$

where v is the drop volume, $We = N^2D^3\rho/\sigma$ is the Weber number representing the ratio of turbulent and surface tension forces, and D is the impeller diameter. For higher viscosity systems, the above correlation was extended (Sathyagal et al. 1996) to give the following correlation

$$\begin{aligned}\Gamma(v)\sqrt{\frac{\rho v}{\sigma}} = 0.422\exp\{-0.2478\ln^2\left[We\left(\frac{v}{D^3}\right)^{5/9}\left(\frac{\mu_c}{\mu_d}\right)^{0.2}\right] \\ +2.155\ln\left[We\left(\frac{v}{D^3}\right)^{5/9}\left(\frac{\mu_c}{\mu_d}\right)^{0.2}\right]\}\end{aligned} \tag{15.35}$$

where μ_d and μ_c refer to dispersed phase and continuous phase viscosities respectively.

15.3.1.4 Cavitational Flow

A cavity will form if the pressure suddenly decreases to a critical value. The cavity will grow; some of the surrounding liquid will evaporate and move into it if the fluid keeps expanding. Such a cavitational flow is very important in ultrasonic and high pressure valve homogenizer (Gopal 1968; Phipps 1985). The cavity will collapse if there is a compression, resulting in an intense shock wave which propagates into the adjacent fluid. These waves cause the droplet to be deformed and disrupted (McClements 1999). Such waves are associated by extremely high pressure and temperature. Although it lasts only very short time and remain in a small local area, it will bring damage to the surfaces of the equipment over a long time, known as '*pitting*' (Gopal 1968; Phipps 1985). Cavitational flow occurs only when the pressure change exceeds a critical value, known as *cavitational threshold* (McClements 1999). For a pure liquid, the cavitational threshold is high therefore it is difficult to form a cavity. If gas bubbles or impurities are presented in the fluid, the cavitational threshold will decrease and consequently it is easier for cavitation.

15.3.1.5 Effect of Non-Newtonian Fluids

The above discussions all assume a constant viscosity. That is, the fluid is Newtonian. However, most of fluids in food industry are non-Newtonian, which have pronounced effects on the breakup of droplets.

For a non-Newtonian fluid, the viscosity depends on the shear rate. For food systems, the liquid usually shows shear thinning behavior, for which the viscosity decreases with increasing shear rate. The liquid may have a yield stress, which means no flow will happen below a certain shear rate; therefore zero shear rate viscosity is infinity. In emulsification equipment, shear rates are different from place to place. Such a difference results in difficulty to predict the behavior of flow and breakup of droplets. For a laminar flow, Eq. (15.20) can be used with apparent viscosity to give

$$d_{\max} = \frac{2\gamma We_{crit}}{G\mu_c'} \tag{15.36}$$

where We_{crit} can be obtained for the corresponding value of $q' = \mu_d' / \mu_c'$.

Often, fluid in food industry showed viscoelastic behavior, which is also shear thinning. The second deviation from Newtonian behavior, elastic deformation, becomes prominent. The relaxation time for disappearance of the elastic stress, τ_{mem} is used to characterize this behavior. For a simple shear in viscoelastic liquid, the critical size for breakup is given by

$$\frac{r_{crit}\mu_d G}{\gamma} = C_1 \tau_{mem} G + C_2 \tag{15.37}$$

where

$$C_1 = \frac{\mu_d}{\left(\mu_c'\right)_0} \tag{15.38}$$

and C_2 is generally between 0.04 and 0.4. When $\tau_{mem} \to 0$, Eq. (15.37) reduces to $We_{crit} = C_2 / q$, corresponding to the result for Newtonian fluid. When $G \to \infty$, Eq. (15.37) leads to

$$r_{crit} \geq C_1 \frac{\tau_{mem}\gamma}{\mu_d} \tag{15.39}$$

This implies that smaller droplets can never be disrupted, no matter how large the shear rate is. For example, $C_1 = 0.01$, $\tau_{mem} = 1$ s, $\gamma = 10$ mN/m and $\mu_d = 10$ cP would give a $r_{crit} > 1$ cm; and this is certainly not an exceptional case. Hence, breakup can become very difficult if the relaxation time τ_{mem} becomes appreciable.

In practice, somewhat smaller droplet can be formed if the flow is not steady, i.e. when dG/dt is large.

15.3.2 Drop Coalescence

In an emulsion forming equipment, the relative motion between droplets caused by turbulence or shear leads to collision between droplets, leading to their coalescence. The drop size of the emulsion is influenced by the rate of coalescence. In the following, we will discuss the evaluation of the rate of drop coalescence due to turbulence.

A colliding drop pair is subjected to interdroplet turbulent and colloidal squeezing force (van der Waals) due to which the intervening continuous phase liquid drains, leading to the coalescence of the pair. On the other hand, the colloidal repulsive forces due to electrostatic and steric interactions counteract the squeezing force thus resulting in a force barrier (Narsimhan 2004) which tends to prevent drop coalescence.

Very little information is available on drop coalescence in high pressure homogenizer during emulsion formation. A contrast matching technique was employed (Tsaine et al. 1996) to infer the drop coalescence in a high pressure homogenizer for surfactant stabilized emulsion. Their results indicated that high surfactant concentration was able to minimize coalescence though extensive coalescence was observed at low surfactant concentration and was found to be higher at higher homogenizer pressures. Drop coalescence in a high pressure homogenizer was inferred (Lobo and Sverika 1997) from the fluorescence of hydrophobic probe that was allowed to transfer between oil droplets. A Monte Carlo simulation was then employed to relate the fluorescence to the coefficient of variation of concentration distribution of the probe in the dispersed phase. Their results were consistent with the earlier results of Tsaine et al. (1996). Drop coalescence was found (Lobo and Sverika 1997) to be insensitive to ionic strength. Narsimhan and coworkers (Mohan and Narsimhan 1997; Narsimhan and Goel 2001) have developed a methodology for the inference of coalescence rates in high pressure homogenizer from the experimental measurement of the evolution of number concentration of droplets to a negative stepchange in homogenizer pressure. The rate of collision between drops of diameter d will depend on the predominant mechanism of collision. For collision due to turbulent shear, the rate of collisions υ_c is given by (Mohan and Narsimhan 1997)

$$\upsilon_c \infty P_h h \tag{15.40}$$

where P_h is the homogenizer pressure and h is the gap thickness in the homogenizer valve. For collision due to turbulence, the rate of collision will depend on whether the drop size is in the inertial or viscous subrange of the universal spectrum and is given by (Mohan and Narsimhan 1997),

$$\upsilon_c \infty P_h^{1/2} h^{1/6}, \quad d \gg \lambda \tag{15.41}$$

$$\upsilon_c \infty P_h^{3/4} h^{1/4}, \quad d < \lambda \tag{15.42}$$

Inference of coalescence rate constants for pure oil in water emulsion in a high pressure homogenizer for different homogenizer pressures gave a functional dependence of $\upsilon_c \infty P_h^{0.722}$ indicating thereby that the predominant mechanism for drop coalescence is turbulence (Mohan and Narsimhan 1997). The effects of homogenizer pressure, droplet size, ionic strength and surfactant concentration on the inferred coalescence rate constant for tetradecane in water emulsion stabilized by sodium dodecyl sulphate (Narsimhan and Goel 2001) are shown in Fig. 15.8. The coalescence rate constant was found to increase with homogenizer pressure (see Fig. 15.8a) as a result of an increase in the turbulent squeezing force of colliding droplet pair at higher homogenizer pressure. The rate constant was also found to be lower for larger drop sizes (see Fig. 15.8b) because of the predominant stabilizing effect of repulsive electrostatic interactions. The coalescence rate constant was found to be insensitive to variations in ionic strength (see Fig. 15.8c).

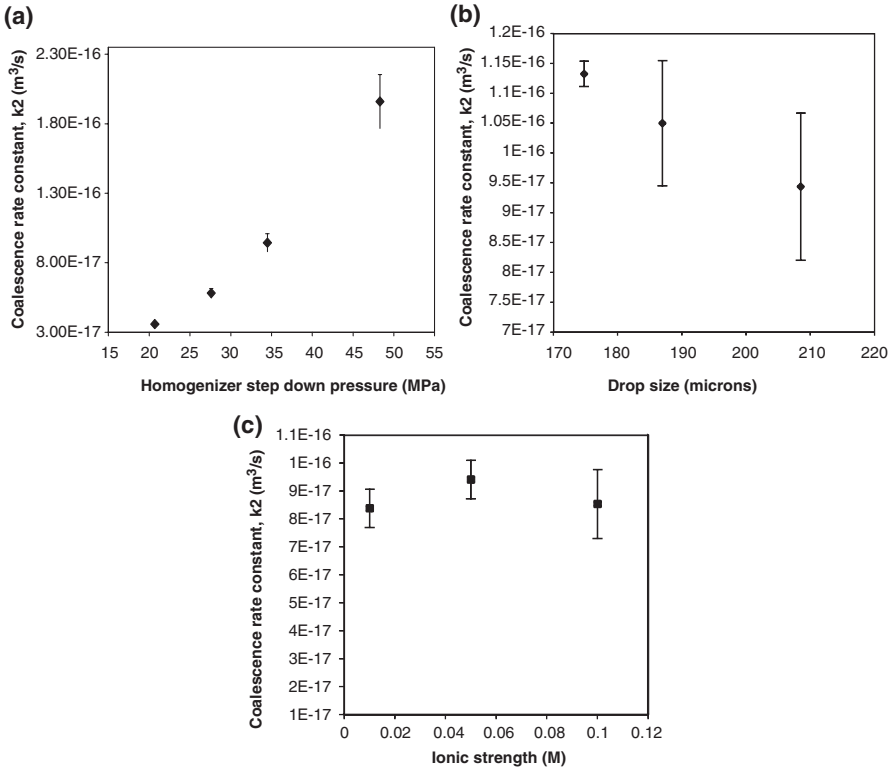

Fig. 15.8 The coalescence rate constant as a function of (**a**) homogenizer step down pressure; (**b**) droplet-size and (**c**) ionic strength

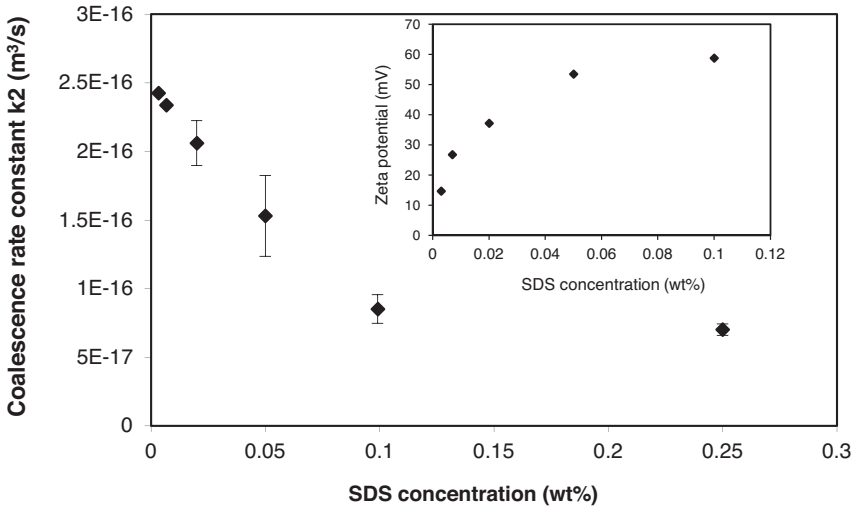

Fig. 15.9 The coalescence rate constant as a function of SDS concentration. Inset: Zeta potential of emulsion as a function of SDS concentration

The coalescence rate constant was found to decrease with an increase in sodium dodecyl sulphate concentration (see Fig. 15.9) leveling off at higher surfactant concentration. This is because of the stabilizing influence due to an increase in the zeta potential of emulsion drops (see inset of Fig. 15.9).

15.3.2.1 Collision of Two Drops

Evaluation of mutual turbulent diffusive flux of two drops, the rate of collisions ν_c is evaluated (Narsimhan 2004) to give,

$$\nu_c = \frac{28\pi}{3}\alpha\varepsilon^{1/3}\left(R_1+R_2\right)^{7/3} n_1 n_2 \quad \left(R_1+R_2\right) \geq \lambda \tag{15.43}$$

$$\nu_c = \frac{4\pi n_1 n_2}{\frac{1}{3}\nu^{1/2}\varepsilon^{-1/2}\left(\frac{1}{\left(R_1+R_2\right)^3}-\frac{1}{\lambda^3}\right)+\frac{3}{7\alpha}\varepsilon^{-1/3}\lambda^{-7/3}} \quad \left(R_1+R_2\right) < \lambda \tag{15.44}$$

where $\alpha = \sqrt{2}$ and $\beta = 1$ are constants.

For equal sized drops of radius R, the above equations reduce to,

$$\nu_c = \frac{28\pi}{3}\alpha\varepsilon^{1/3}\left(2R\right)^{7/3} n_0^2 \tag{15.45}$$

$$\nu_c = \frac{4\pi n_0^2}{\frac{1}{3}\nu^{1/2}\varepsilon^{-1/2}\left(\frac{1}{(2R)^3} - \frac{1}{\lambda^3}\right) + \frac{3}{7\alpha}\varepsilon^{-1/3}\lambda^{-7/3}} \tag{15.46}$$

where n_0 is the number concentration of drops.

The time scale of drop collision, τ_{coll} for equal sized drops can be defined as,

$$\tau_{coll} = \frac{n_0}{\nu_c} \tag{15.47}$$

where ν_c is given by Eq. (15.13) or (15.16) and n_0, the number of droplets per unit volume is given by

$$n_0 = \frac{3\phi}{4\pi R^3} \tag{15.48}$$

where ϕ is the dispersed phase fraction and R is the mean droplet radius. The time scale of drop collision, τ_{coll} for equal sized drops can be defined as,

$$\tau_{coll} = \frac{n_0}{\nu_c} \tag{15.49}$$

where ν_c is given by Eq. (15.45) or (15.46) and n_0, the number of droplets per unit volume is given by

$$n_0 = \frac{3\phi}{4\pi R^3} \tag{15.50}$$

where ϕ is the dispersed phase fraction and R is the mean droplet radius.

For non deformable spherical particles, the drainage of continuous phase liquid between two colliding particles of size d and d' is given by Taylor's equation (Narsimhan 2004).

$$\frac{dh}{dt} = \frac{2hF}{3\pi\mu}\left(\frac{1}{d} + \frac{1}{d'}\right)^2 \tag{15.51}$$

where h is the surface to surface distance between the drops and F is the interaction force between the two emulsion droplets.

By convention, the interaction force F is positive if repulsive and negative if attractive. For drops of equal size d, Eq. (15.51) becomes

$$\frac{dh}{dt} = \frac{8hF}{3\pi\mu d^2} \tag{15.52}$$

In a turbulent flow field, the droplet pair is subjected to random fluctuating turbulent force with mean force $\bar{F}$ which will try to squeeze the colliding drop pair towards each other thus promoting coalescence. Van der Waals attractive force between the two drops would also promote coalescence. On the other hand, the electrostatic repulsive force between the two drops would tend to slow down the film drainage.

The mean turbulent force, $\bar{F}$ is given by (Narsimhan 2004)

$$\bar{F} = \frac{\pi d^2 \rho}{4} \overline{u^2}(d) \tag{15.53}$$

where $\overline{u^2}(d)$ is the mean square turbulent velocity fluctuation between the centers of the colliding droplet pair separated by a distance d.

For local isotropy, when $d \geq \lambda$ (inertial subrange) the mean square velocity fluctuation is given by Eq. (15.22).

The mean turbulent force $\bar{F}$ is therefore given by

$$\bar{F} = \frac{\pi}{2} \rho \varepsilon^{2/3} d^{8/3} \tag{15.54}$$

For local isotropy, when $d \leq \lambda$ (viscous subrange) the mean square velocity fluctuation is given by Eq. (15.23).

The mean turbulent force $\bar{F}$ is therefore

$$\bar{F} = \frac{\pi}{4} \frac{\rho^2 d^4 \varepsilon}{\mu} \tag{15.55}$$

One can estimate the timescale of film drainage for a colliding drop pair by neglecting the effect of colloidal forces to give,

$$t_{dr} = \frac{h}{dh/dt} = \frac{3\pi\mu d^2}{8\bar{F}} \tag{15.56}$$

For relatively large drop sizes (10–100 μm) and relatively low intensity turbulent flow fields, the timescale of drop collisions (as given by Eq. (15.47)) is much larger than the timescale of coalescence of the drop pair (Eq. (15.49)). Consequently, the rate of coalescence can be expressed as, Rate of coalescence = rate of collision × coalescence efficiency.

15.3.2.2 Models for Coalescence Efficiency

Coulaloglou and Tavlarides (1977) recognized the probabilistic nature of the coalescence process. They suggested that the force which compresses the drops must act for a sufficient time that the intervening film drains to a critical thickness so that the film ruptures and coalescence will take place. Consequently, the contact time τ

between colliding drops must exceed the coalescence time t of the drops. For contact time τ that is normally distributed, the coalescence efficiency is given by,

$$\eta = \exp\left(-\bar{t} / \bar{\tau}\right) \tag{15.57}$$

where $\bar{t}$ and $\bar{\tau}$ are averages.

The contact time is estimated by the time two drops of size d_1 and d_2 will stay together and is proportional to the characteristic period of velocity fluctuation of an eddy of size $d_1 + d_2$. For drops in the inertial subrange,

$$\bar{\tau} \sim \frac{\left(d_1 + d_2\right)^{2/3}}{\varepsilon^{1/3}} \tag{15.58}$$

Therefore, the coalescence efficiency can be written as,

$$\eta\left(d_1, d_2\right) = \exp\left[-\frac{c\mu\rho\varepsilon}{\sigma^2}\left(\frac{d_1 d_2}{\left(d_1 + d_2\right)}\right)^4\right] \tag{15.59}$$

Das et al. (1987) considered the stochastic nature of drop coalescence by considering the random fluctuations of turbulent force acting on the colliding pair of droplets. They described the force as Gaussian white noise superimposed on a mean turbulent force, i.e.

$$F = \bar{F} - \delta T_f^{1/2} \zeta\left(t\right) \tag{15.60}$$

where δ is the standard deviation of the fluctuating force, T_f is the timescale of force fluctuation and $\zeta(t)$ is white noise. As a result, the drainage equation for the continuous phase film became a stochastic differential equation,

$$\frac{dh}{dt} = \frac{8h}{3\pi\mu\, d^2}\left(\bar{F} - \delta T_f^{1/2} \zeta\left(t\right)\right) \tag{15.61}$$

Because of the random nature, the thickness of the draining film will be random thus reaching the critical film thickness of rupture at different times. Das et al. (1987) formulated the Fokker Planck equation corresponding to the above stochastic equation to obtain the mean coalescence time of the drop pair in terms of the characteristics of the turbulent random force. As expected, the average coalescence time was smaller (larger coalescence rate) for larger turbulent force. Interestingly, their model predicted higher coalescence efficiency for higher continuous phase viscosity. Muralidhar et al. (1988) extended this analysis to band limited noise and considered both non-deformable and deformable colliding drop pair. When the ratio of the characteristic time of force fluctuation and timescale of film drainage becomes large, the turbulent force can be considered to be a random variable and Coulaloglou

and Tavlarides (1977) analysis is then applicable for the prediction of coalescence frequency.

For sufficiently small drop sizes (0.1 to a few μm) and high-intensity turbulent flow fields, the timescales of collision and coalescence are comparable. Therefore, the rate of coalescence cannot be expressed by collision efficiency. Narsimhan (2004) visualized drop coalescence as consisting of two steps, namely, formation of a doublet due to drop collisions, followed by drop coalescence due to rupture of thin liquid film separating the drops. The evolution of number concentration is given by,

$$\frac{dn_1}{dt} = -k_1 n_1^2 + k_d n_d \tag{15.62}$$

$$\frac{dn_d}{dt} = k_1 n_1^2 - \left(k_d + k_2\right) n_d \tag{15.63}$$

$$\frac{dn_c}{dt} = k_2 n_d \tag{15.64}$$

where n_1, n_d and n_c are the number concentration per unit volume of the monomer, the doublet and coalesced droplet respectively, k_1 is the rate constant for the formation of doublet, k_d is the rate of dissociation of the doublet and k_2 is the rate of coalescence of the doublet. These have to be solved with the initial condition,

$$t = 0, \quad n_1 = n_0, \quad n_d = 0, \quad n_c = 0 \tag{15.65}$$

The rate constant k_1 for the formation of doublet can be taken as the rate constant for the rate of collisions as given by,

$$k_1 = \frac{\nu_c}{n_0^2} \tag{15.66}$$

where ν_c, the rate of collisions per unit volume is given by Eqs. (15.43) and (15.44) for inertial and viscous subranges respectively.

Once a doublet is formed, it is subjected to random turbulent force fluctuation. The net turbulent force acting on the doublet at the time of collision is given by (Narsimhan 2004),

$$F = -\left\{\bar{F} - \delta T_f^{1/2} \zeta\left(t\right)\right\} \tag{15.67}$$

since the colloidal interaction force at the time of doublet formation is negligible. In the above equation, $\bar{F}$ is the mean turbulent force, δ is the standard deviation, T_f is the timescale of force fluctuation and $\zeta(t)$ is white noise. It is to be noted that the average turbulent squeezing force is attractive. In order for the doublet to separate, the fluctuating force should overcome the mean force $\bar{F}$. The fluctuating force acting on the doublet is modeled as a Poisson process, i.e. a force of magnitude δ

(equal to $\bar{F}$) acts on the doublet at random times with a decay timescale T_f Narsimhan (2004) has evaluated the rate of dissociation of the doublet k_d (inverse of the average dissociation time of the doublet) as,

$$k_d = \frac{1}{0.37T_f} \tag{15.68}$$

T_f being the decay time of turbulent force fluctuation.

The evaluation of the rate of coalescence of drops in a doublet k_2 involves the determination of the average rupture of continuous phase film separating the droplets in the doublet that are exposed to turbulent pressure fluctations. Narsimhan (2004) adopted the same approach as that of Das et al. (1987) and Muralidhar and Ramkrishna (1986) in expressing the thickness of the film by a stochastic differential equation. Unlike the earlier investigators, Narsimhan (2004) also considered colloidal van der Waals and electrostatic interactions between the droplets in the evaluation of film drainage. Therefore, the net force of interaction experienced by the droplet pair is the sum of the turbulent and colloidal forces. Because of the random nature of the turbulent force, the surface to surface distance $h(t)$ can be considered to be a stochastic process. The net interaction force F is given by (Narsimhan 2004):

$$F = \bar{F} - F_c - \delta T_f^{1/2} \zeta\left(t\right) \tag{15.69}$$

where $\bar{F}$ is the mean turbulent force given by Eqs. (15.54) and (15.55) and F_c is the colloidal interaction between the droplets due to Van der Waals and electrostatic forces.

$$F_c = F_{VW} + F_{DL} \tag{15.70}$$

where F_{VW} and F_{DL} refer to the van der Waals and double layer interactions, respectively.

The last term in Eq. (15.69) refers to the turbulent fluctuating force which is explained later in this section. It is to be noted that the hydrodynamic interaction between the two colliding drops is neglected in this analysis. Such an assumption is indeed reasonable for sufficiently small droplets. The Van der Waals interaction is given by (15.14)

$$F_{VW} = -\frac{A_H R_1 R_2}{6h^2\left(R_1 + R_2\right)} \tag{15.71}$$

where A_H is the Hamaker constant.

Some surfactants are ionic; all proteins have acidic (–COOH ⇌ COO^- + H^+) and basic ($NH_2 + H^+ \rightleftharpoons NH_3^+$) groups therefore are capable of being ionized. Such charged molecules adsorbed at the interface form a charged layer at the oil-liquid

interface. This charged layer results in an electrical double layer near the droplet surface. When two droplets move to each other, the potential between the double layers overlap resulting repulsive force to prevent the two droplets getting closer (Hiemenz and Rajagopalan 1997). A schematic of the double layer and potential profile is shown in Fig. 15.10.

The electrostatic force of interaction F_{FP} per unit area between two plates separated by a distance h is then given by,

$$F_{FP} = 2kTn_o\left[\cosh Y_m - 1\right] \tag{15.72}$$

where Y_m is the dimensionless midpoint potential defined as,

$$Y_m = \frac{ze\psi_m}{kT} \tag{15.73}$$

In the above equation, z is the valence number of the electrolyte, e is the elementary charge and ψ_m is the midpoint potential which is to be obtained from the solution of Poisson Boltzmann equation.

Using Derjaguin approximation, the interaction force $F_{DL}(h)$ between two droplets of radius R separated by a surface to surface distance h can be obtained by integration to give,

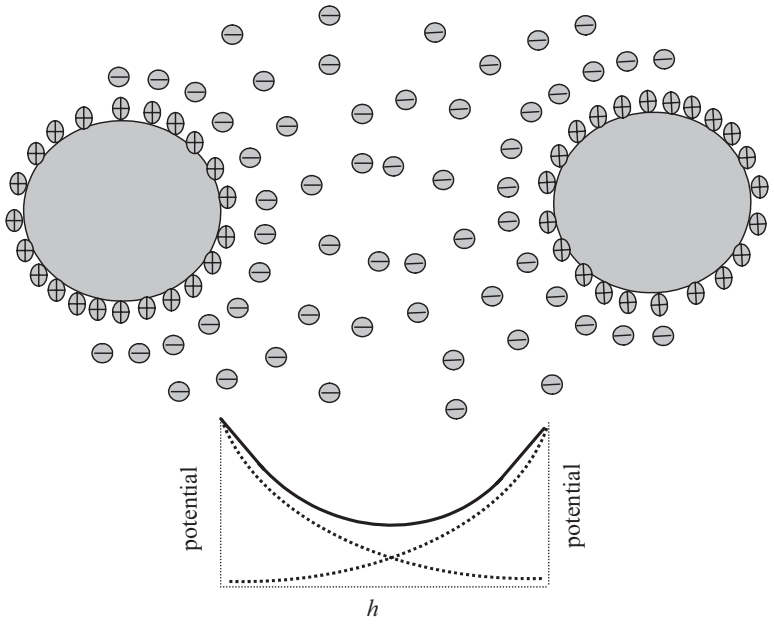

Fig. 15.10 A schematic of electrical double layer and the potential profile between two charged droplets

$$F_{DL}(h) = \pi R \int_{h}^{\infty} F_{FP}(x)\, dx \tag{15.74}$$

Narsimhan (2004) has analyzed the film drainage accounting for interdroplet turbulent and colloidal forces to evaluate the mean rupture time of the film and hence the rate of coalescence k_2 (inverse of the mean rupture time). In addition, his analysis also gave the second moment of rupture time distribution. The predicted average drop coalescence time was found to be smaller for larger turbulent energy dissipation rates, smaller surface potentials, larger drop sizes, larger ionic strengths and larger drop size ratio of unequal size drop pair. The predicted average drop coalescence time was found to decrease whenever the ratio of average turbulent force to repulsive force barrier becomes larger. The calculated coalescence time distribution was broader with a higher standard deviation at lower energy dissipation rates, higher surface potentials, smaller drop sizes and smaller size ratio of unequal drop pair. The variation of average coalescence time with energy dissipation rate is shown in Fig. 15.11.

The average coalescence time decreases exponentially as the energy dissipation rate increases (Fig. 15.11). The ratio of the average turbulent force to the colloidal barrier force versus energy dissipation rate is also shown in the same figure. It is

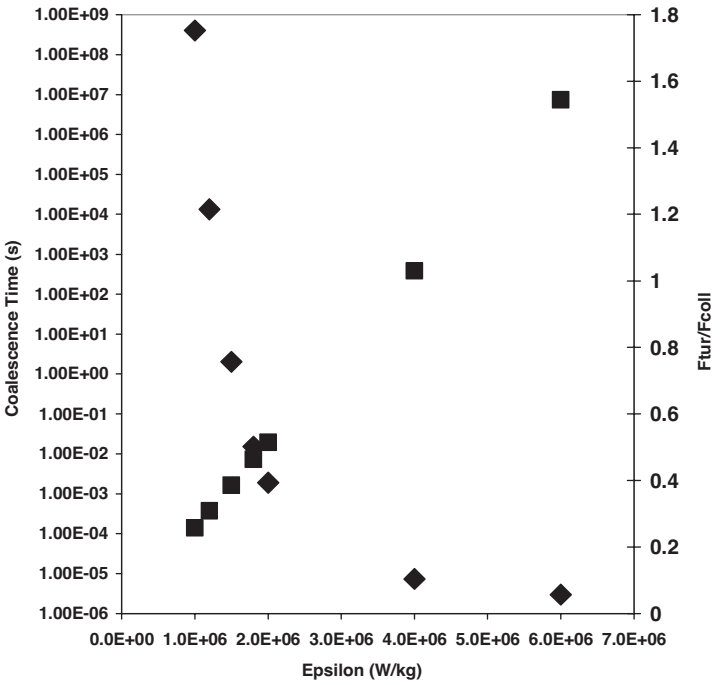

Fig. 15.11 The coalescence time as a function of energy dissipation

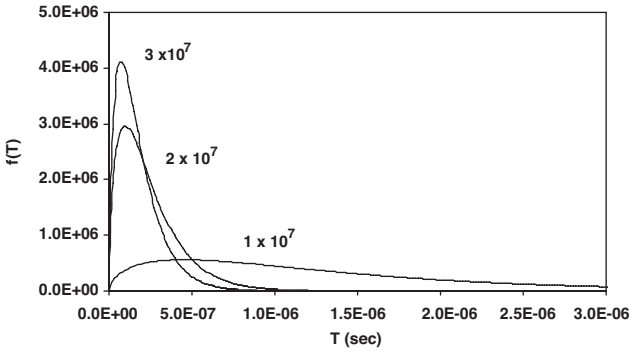

Fig. 15.12 The coalescence time distribution as a function of turbulent density

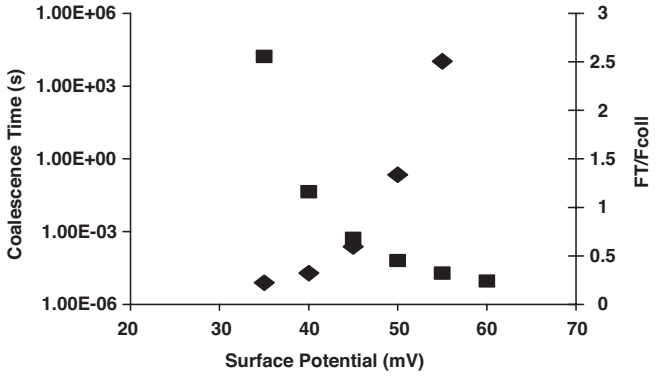

Fig. 15.13 The average coalescence time as a function of surface potential

interesting to note that the coalescence time decreases dramatically as this ratio increases. The coalescence time distribution (see Fig. 15.12) becomes broader with a larger standard deviation at lower turbulent intensity. The average coalescence time was found to increase dramatically (see Fig. 15.13) with the surface potential. For example, the average coalescence time increases from ~10^{-5} to ~10^{3} s as the surface potential increases from 35 to 55 mV. This behavior is due to the increase in the colloidal force barrier due to an increase in the electrostatic repulsion. Similarly, the average coalescence time was found to decrease with an increase in ionic strength (see Fig. 15.14) as a result of smaller electrostatic repulsion caused by the compression of the double layer. The model predictions of average coalescence rate constants for tetradecane-in-water emulsions stabilized by sodium dodecyl sulphate (SDS) in a high pressure homogenizer agreed fairly well with the values inferred from experimental data as reported by Narsimhan and Goel (2001) at different homogenizer pressures and SDS concentrations (Fig. 15.15).

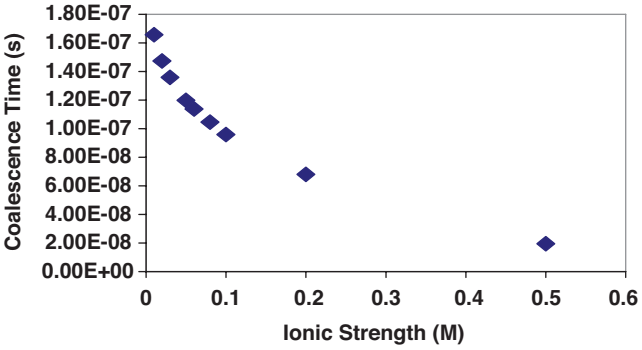

Fig. 15.14 The average coalescence time as a function of ionic strength

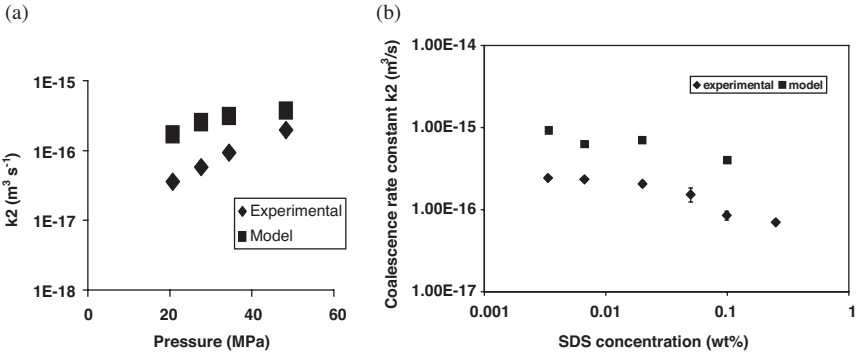

Fig. 15.15 Comparison of predicted and experimental drop coalescence rate constants in a high pressure homogenizer. The model predictions are made for (**a**) different homogenizer pressures $\bar{R} = 1.951 \times 10^{-7}$ m, $\sigma = 3.03 \times 10^{-8}$ m, $I = 0.05$, $\varepsilon = 7.526 \times 10^{7}$ W/kg, $\psi_0 = 63.9$ mV, ◆: experimental; ■: model prediction. (**b**) Different SDS concentrations, $\bar{R} = 2.32 \times 10^{-7}$ m, $\sigma = 3.203 \times 10^{-8}$ m, $I = 0.1$, $\varepsilon = 4.82 \times 10^{7}$ W/kg, ◆: experimental; ■: model prediction. (Reproduced from Narsimhan 2004)

15.3.3 Role of Surfactants and Proteins on Emulsion Formation

Surfactants and proteins reduce interfacial tension by adsorbing onto the oil-water interface thereby reducing the energy required for emulsion formation. More importantly, the surfactants prevent drop coalescence by various mechanisms thus providing shelf life to such systems. The reduction in interfacial tension is denoted as the surface (interfacial) pressure π defined as,

$$\pi = \gamma_0 - \gamma \tag{15.75}$$

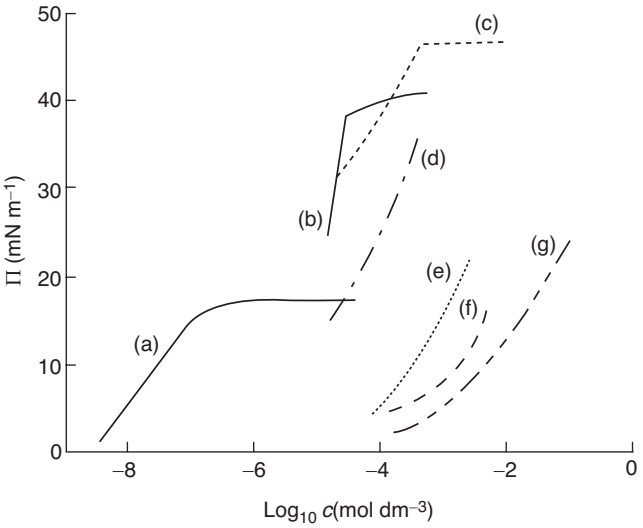

Fig. 15.16 Plot of surface pressure π against logarithm of bulk concentration c for lysozyme and various small-molecule surfactants adsorbing at the oil-water interface: (a) lysozyme (toluene-water), (b) Span 80 (n-octane-water), (c) actylphenoxyethoxyethanol (iso-octane-water), (d) SDS (n-heptane-water), (e) isosorbide monolaurate (n-decane-water), (f) SDS (triglyceride-water), and (g) dodecanoic acid (n-hexane-water) (Fisher et al. 1988)

where γ_0 and γ refer to the interfacial tension of pure oil-water interface and interface with adsorbed surfactant respectively. Typical variation of interfacial tension with bulk concentration is shown for different types of surfactants and proteins in Fig. 15.16. As can be seen from the figure, small molecular weight surfactants are more efficient in lowering the interfacial tension than proteins and are therefore necessary in food formulations. The surface pressure can be as high as 50 mN/m (interfacial tension as low as 22 mN/m) for surfactants. Proteins, however, do not reduce the interfacial tension by more than 20 mN/m. As will be discussed later, proteins are essential for providing long term stability. At low concentrations, the surface pressure is given by

$$\pi = \Gamma RT \tag{15.76}$$

where the surface concentration Γ is related to bulk concentration c by Gibbs adsorption equation,

$$\Gamma = -\frac{c}{RT}\frac{d\gamma}{dc} \tag{15.77}$$

At concentrations above critical micelle concentration (cmc), the surface concentration remains constant since the oil-water interface is covered by a monolayer of surfactant.

The adsorption isotherms for macromolecules are much more complicated. Singer (1948) applied a simple lattice model to describe adsorption of macromolecules at interfaces. This model assumed that all the segments of protein molecule adsorbed at the interface in the form of trains. The model is able to predict the isotherm for β casein at air-water interfaces up to a surface pressure of 8 mN/m. However, the experimental data for the globular proteins BSA and lysozyme do not agree with Singer's model since the proteins do not adsorb in the form of trains. The isotherms at oil-water interface were found to be more expanded compared to Singer's model for both the proteins. Frisch and Simha (1956) allowed for the adsorption of segments in the form of both trains and loops to modify Singer's model to give the following expression for the surface pressure,

$$\frac{\pi a_0}{kT} = \left\{\left(y-1\right)z/2y\left(1-x^{-1}\right)\right\}\ln\left\{1-\left[2p\theta\left(1-x^{-1}\right)/z\right]\right\} - \ln\left(1-p\theta\right) \qquad (15.78)$$

where π is the surface pressure, a_0 is the limiting close packed area per segment, k is the Boltzmann constant, T is the temperature, x is the total number of segments of the molecule, z is the coordination number of the lattice and the surface coverage θ is defined as, $\theta = a_0/a$, a being the average area occupied by a segment, y is the total number of train segments directly in contact with the interface and $p = y/x$.

A globular protein molecule in aqueous solution tends to assume a tertiary structure in which most of the hydrophobic functional groups are buried inside the protein molecule and the hydrophilic functional groups are exposed to the aqueous medium since such a conformation is energetically most favorable. The extent of penetration and subsequent unfolding of the molecule depends on the surface pressure and segment-segment interactions. Uraizee and Narsimhan (1991) proposed a two dimensional lattice model which accounts for entropy and enthalpy of mixing of the adsorbed segments at the interface as well as electrostatic interactions because of the presence of electrical double layer in the vicinity of adsorbed layer of protein. They also accounted for the dependence of extent of unfolding of the molecule on the surface concentration. In other words, their model postulated that the adsorbed protein molecule completely unfolded at very low surface concentrations (or, equivalently, surface pressures) with the extent of unfolding decreasing at higher surface concentrations. Even though this model is more complex and therefore has more parameters; it is more realistic in that it accounts for functional dependence of unfolding on surface pressure as well as electrostatic interactions. This model, however, accounts only for monolayer adsorption of protein at air-water interface. Douillard and Lefebvre (1990) proposed a phenomenological model accounting both for unfolding of the protein molecule in the first adsorbed layer as well as the formation of a second adsorbed layer. All of these models have many parameters, which limit their applications.

In fact, adsorption kinetics is more important for the efficiency of emulsifiers. As shown in Fig. 15.3, after a droplet rupture occurs, the rate of emulsifier adsorption is a critical factor determining its fate to be stably existing or coalescence. Generally speaking, surfactants adsorb much faster than macromolecules such as proteins.

Consequently, surfactants are favorable to breakup droplets. However, protein has its advantage in providing better rheological properties thus emulsion stability (will be discussed later). Adsorption of surfactants is usually diffusion controlled, whose adsorption rate is mainly determined by the bulk concentration. For proteins, molecules have to overcome an energy barrier before it reaches the interface. Electrical double layer is the main source of energy barrier. The surface potential comes from the charges in the adsorbed protein layer and the energy barrier comes from the interaction between the surface charge and the double layer. More charge every single molecule brings, higher energy barrier. When the pH of the solution is close to pI of the protein, the molecules bring fewer charges. This is favorable for protein adsorption. However, close to pI, the surface potential is lower therefore the double layer repulsive force is weaker, which is favorable for droplet coalescence. Consequently, the adsorption rate has to compromise with the emulsion stability.

15.3.3.1 Interfacial Dilatational and Shear Rheology

Interfacial rheology is the relationship between the deformations of an interface and accompany forces as a function of time. Dilatational deformation refers to that the interface area changes while the interface shape kept. Shear deformation refers to that the interface area is constant while the surface shape is distorted. The relationship between interfacial rheology and the emulsion/foam stability gained much attention in recently years. There is experimental evidence to show that an increase in stability is attained with an increase in interfacial rheology (Bos and van Vliet 2001).

The surfactants and protein molecules at the interface are shown in Fig. 15.17. There are no structural changes in surfactant molecules, nor intermolecular interaction in surfactants. For proteins, the molecules adsorbed at the interface and undergoing conformational changes. A flexible protein, e.g. casein monomer, adsorbs to the interface to give an entangled monolayer of flexible chains having sequences of segments in direct contact with surface ('trains') and others protruding into aqueous phase ('loops' or 'tails') (Dickinson 2001). Hard protein, e.g. β-lactoglobulin, forms

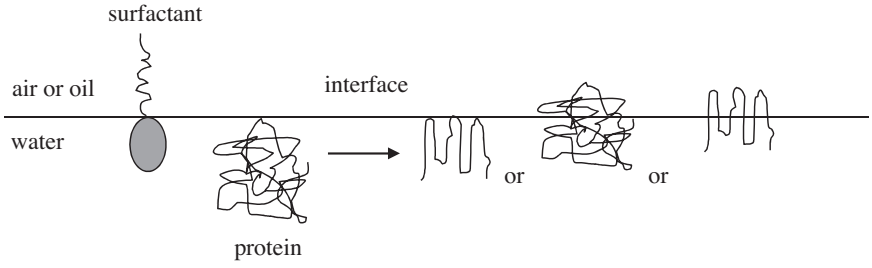

Fig. 15.17 Conformation of a low molecular weight surfactant a protein molecule at a fluid-fluid interface (not to scale). The two drawings on the right apply to oil-water interface. (Adapted from Bos and van Vliet 2001)

a rather dense and thin adsorbed layer (Atkinson et al. 1995). At the time the protein molecules adsorb, the protein layer can be regarded as a closed packed monolayer of deformable particles (De Feijter and Benjamins 1982). Following the molecules adsorption, the protein molecules unfold and form a 2-D gel like layer. The intermolecular interaction and covalent crosslink strengthen the gel-like structure. Such a gel-like layer showed a viscoelastic behavior.

The interfacial shear viscosities are much higher than those of surfactants. The interfacial shear viscosity and shear modulus of some proteins are shown in Table 15.3. Among proteins, the globular proteins showed much higher interfacial shear viscosities than those of flexible proteins. Murray and Dickinson (1996) observed a large time before interfacial shear viscosity starts to increase when protein concentration is low. This may suggests that a certain level of protein molecules is necessary to form a gel-like layer. Heating results in higher interfacial shear viscosity, probably due to the formation of cross-links between molecules (Dickinson and Matsumura 1994).

According to Djabbarah and Wasan (1982), the magnitude of interfacial shear viscosity and elasticity for surfactants at air-water interface is several orders lower than that of dilatational viscosity and elasticity under the same conditions. Unlike shear rheology, the dilatational rheological properties are not sensitive to protein type and molecular structure (Murray and Dickinson 1996).

15.3.3.2 Mechanisms of Stabilization

Surfactant molecules tend to stabilize the oil-water interface by two distinct mechanisms, namely, (1) Marangoni effect and (2) interfacial rheology. Large globules are stretched in the form of cylindrical threads before they are broken into smaller fragments. In addition, coalescence of two colliding droplets depends on the stability of intervening thin liquid film of continuous phase. Consequently, the ability of surfactant to provide stability to oil-water interface influences both drop breakup as well as coalescence. The mobility of oil-water interface with adsorbed layer of surfactant leads to regions of depleted and concentrated surfactants which results in a gradient

Table 15.3 Interfacial shear viscosity and shear modulus of various proteins for n-tetradecane-water interface after an adsorption of 24 h (Kokelaar and Prins 1995)

Protein	η^s (mN·s/m)	G^s (mN/m)
β-Casein	0.5	0.1
α_{s1}-Casein	4.0	0.3
Na-caseinate	7.4	0.6
Gelatin	120	0.6
α-Lactalbumin	170	–[a]
κ-Casein	180	5.0
Lysozyme	630	23.0
β-Lactoglobulin	1200	–
Myosin	2400	–

[a]–, not determined

of interfacial concentration of surfactant. This, in turn, results in a gradient of interfacial tension. This gradient causes an interfacial stress, known as Marangoni stress, that opposes the mobility of the interface thus providing stability as depicted schematically in Fig. 15.18. Of course, the gradient of surfactant interfacial concentration is reduced by (1) spreading of surfactant molecules at the interface due to surface diffusion from regions of higher concentration to regions of lower concentration and (2) adsorption of surfactant from the bulk onto the surface in regions of lower surface concentrations. As pointed out above, proteins provide interfacial shear and dilatational rheology to the oil-water interface. Consequently, adsorption of proteins provides resistance to interfacial mobility due to shear viscosity and resistance to interfacial deformation due to dilatational viscosity.

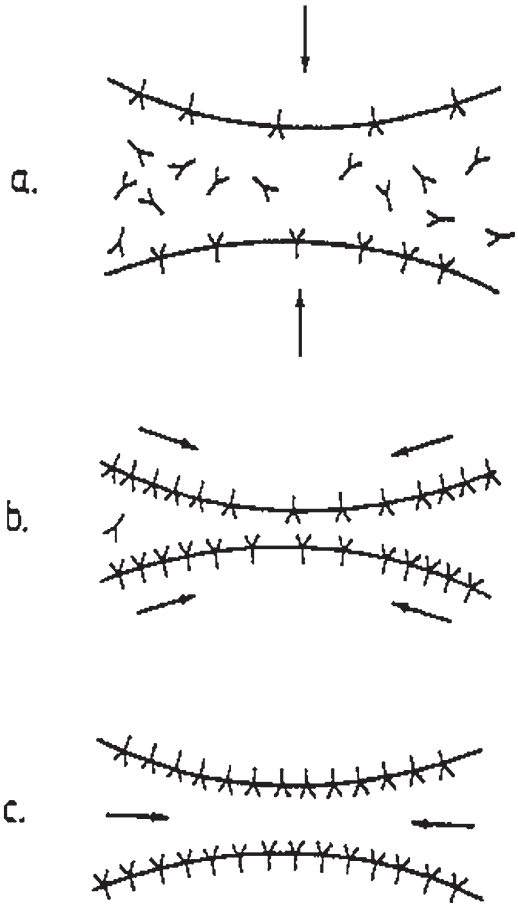

Fig. 15.18 Diagram explaining the Gibbs-Marangoni effect for two approaching droplets during emulsification. Surfactant molecules are depicted by Y (Walstra 1993) (**a**) thinning of the film (**b**) interfacial mobility of surfactant resulting in a gradient of surface concentration (**c**) equalization of gradient of surface concentration by adsorption from the bulk

Consider the stability of an equilibrium thin plane parallel film between two droplets that is stabilized by a mixture of proteins and food emulsifiers. One can characterize the stability of an equilibrium film by analyzing the growth of perturbations of different wavenumbers. It is customary to consider the growth of an asymmetric periodic disturbance of a fixed wavenumber of the form,

$$f\left(x,t\right)=f_0\exp\left(ikx+\beta t\right) \tag{15.79}$$

where k is the wavenumber and β is the growth coefficient. The growth coefficient can be evaluated by solving the velocity fields generated by the imposed disturbance subject to the following boundary conditions:

Kinematic boundary condition:

$$v_y\left(x,h,t\right)=\frac{\partial f}{\partial t} \tag{15.80}$$

Normal stress boundary condition:

$$p'\left(h\right)+\Pi'\left(h\right)f_0+2\mu_d\left(\frac{dv_{y,d}^{'}\left(h\right)}{dy}\right)-2\mu\left(\frac{dv_y^{'}\left(h\right)}{dy}\right)=\sigma f_0k^2 \tag{15.81}$$

where the first term is the imposed pressure disturbance, the second term is the change in the disjoining pressure because of imposed disturbance, the third and fourth terms refer to the normal stress at the interface due to flow in the drop and continuous phases respectively.

Shear stress boundary condition:

$$\begin{aligned}&\mu\left(\frac{\partial v_y}{\partial x}+\frac{\partial v_x}{\partial y}\right)_{y=h}-\mu_d\left(\frac{\partial v_{y,d}}{\partial x}+\frac{\partial v_{x,d}}{\partial y}\right)_{y=h}\\&=\frac{\partial\sigma}{\partial\Gamma}\frac{\partial\Gamma}{\partial x}+\int_{-\infty}^{t}\left(G_d\left(t-t'\right)+G_s\left(t-t'\right)\right)\frac{\partial^2v_x^0}{\partial x^2}dt'\end{aligned} \tag{15.82}$$

where the first two terms on the left hand side refer to the shear stress at the interface due to flow in continuous and dispersed phases respectively, the first term on the right hands side is the Marangoni stress and the second term is the stress due to interfacial dilatational and shear rheology. In the above equation, $G(t)$ and $G_d(t)$ refer to the surface shear and surface dilatational relaxation modulus respectively. For a Maxwell model, they are given by,

$$G_d\left(s\right)=\frac{\kappa}{\lambda_d}\exp\left(-\frac{s}{\lambda_d}\right) \tag{15.83}$$

$$G_s(s) = \frac{\mu_s}{\lambda_s} \exp\left(-\frac{s}{\lambda_s}\right) \tag{15.84}$$

where κ and μ_s are dilatational and shear viscosities respectively. λ_d and λ_s are relaxation times defined as

$$\lambda_d = \frac{\kappa}{g_d} \tag{15.85}$$

$$\lambda_s = \frac{\mu_s}{g_s} \tag{15.86}$$

where g_d and g_s are dilatational and shear elasticity respectively. In order to evaluate the Marangoni stress, one needs to solve for the interfacial concentration gradient from the continuity equation for the surfactant in the bulk and the following surfactant balance at the air-liquid interface,

$$-D\left.\frac{\partial c}{\partial y}\right|_{y=h} = \frac{\partial}{\partial x}\left(\Gamma v_x^0\right) - D_s \frac{\partial^2 \Gamma}{\partial x^2} + \left(\frac{\partial \Gamma}{\partial c}\right)_0 \frac{\partial c}{\partial t} \tag{15.87}$$

where D and Γ are the diffusion coefficient and surface concentration of surfactant respectively, v_x^0 is the interfacial velocity of the film and D_s is the surface diffusion coefficient. If the resistance to adsorption from the subsurface to the surface is much smaller than the diffusional resistance, the subsurface can be assumed to be in equilibrium with the surface as given by $\left(\frac{\partial \Gamma}{\partial c}\right)_0$.

Narsimhan and Wang (2005) have solved for the growth coefficient of imposed disturbance as a function of wavenumber for different interfacial viscoelasticity of a foam film. Typical plots of β versus k are shown in Fig. 15.19. $\beta_{\max}$ for a mobile interface is the largest, while that for an immobile interface is the smallest. $\beta_{\max}$ increases with decreasing interfacial rheological properties. This shows that rheological properties increased the film stability. Effects of λ_d and λ_s are shown in Fig. 15.20. λ_d and λ_s also have symmetric position in equations, therefore only one of them (denoted as λ) is varied here. From Fig. 15.20, for any specified μ_s and κ, when λ is large enough, the film behaves as that with a mobile interface. For intermediate range of λ, $\beta_{\max}$ decreases with the decrease of λ. When λ decreases to a small enough value, $\beta_{\max}$ decrease to a constant value and is no longer dependent on λ. This intermediate range shifts to smaller λ when κ and μ_s decrease.

In order to ascertain the importance of surface shear and dilatational rheological properties on stability of protein stabilized film, calculations of $\beta_{\max}$ for different film thickness was carried out for a film stabilized by β-lactoglobulin at pH 7 and ionic strength of 0.02. Based on high frequency limits of these surface shear and dilatational rheological properties and under the assumption of negligible Marangoni

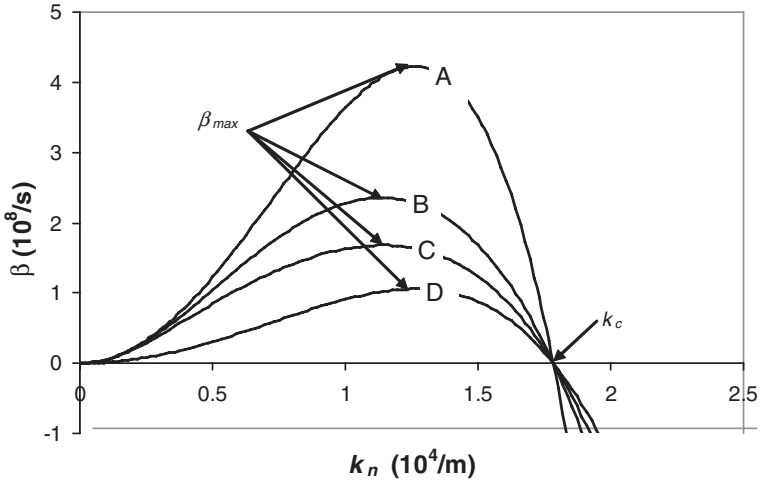

Fig. 15.19 Growth coefficient versus wave number at a given film thickness. A–D are for mobile, viscoelastic (κ = 0.1 N · s/m), viscoelastic (κ = 1 N · s/m) and immobile interface respectively. Parameters for viscoelastic interface are: $\mu_s = 0.4$ N · s/m, $\lambda_d = 10$ s and $\lambda_s = 4$ s; other parameters are $\mu = 10$ Pa · s, $\sigma = 50$ mN/m, $A = 10^{-20}$ J and $h = 10^{-7}$ m

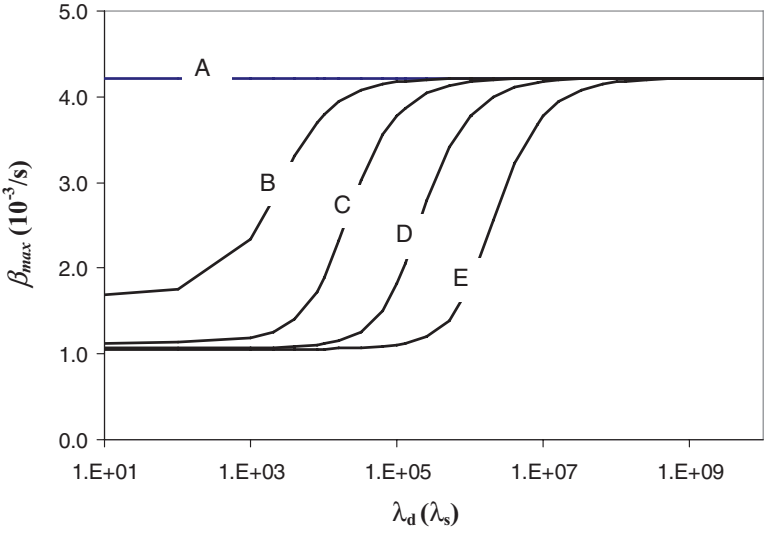

Fig. 15.20 β_{max} versus λ_d for different κ and μ_s. Parameters are $\mu = 5$ Pa · s, $\sigma = 50$ mN/m, $A = 10^{-20}$ J and $h = 10^{-7}$ m. κ and μ_s values for different curves are A: 0 and 0; B: 1×10^{-3} and 4×10^{-4} N · s/m; C: 1×10^{-2} and 4×10^{-3} N · s/m; D: 1×10^{-1} and 4×10^{-2} N · s/m and E: 1 and 0.4 N · s/m respectively

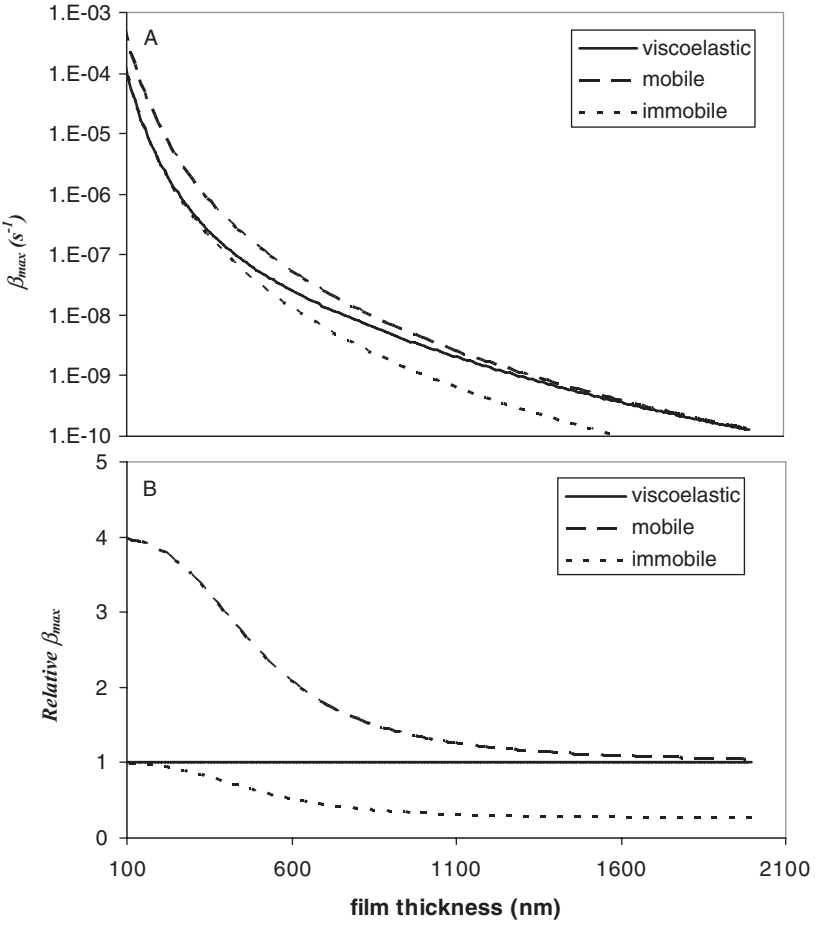

Fig. 15.21 (**a**) Actual and (**b**) relative maximum growth coefficient (normalized by maximum growth coefficient for viscoelastic case) of a β-lactoglobulin stabilized thin film at a solid surface. $A = 10^{-20}$ J, $\gamma = 50$ nm/m. For the actual viscoelastic surface, $\kappa_s = 12.9$ mN · s/m, $G_s = 18.8$ mN/m, $\mu_s = 3.9$ mN · s/m, $\eta = 103$ mN/m

effect, β_{max} was calculated for different film thicknesses and compared with the corresponding values for mobile and immobile films (see Fig. 15.17). Figure 15.17 also gives the relative values of β_{max} for different film thickness. It is interesting to note that β_{max} values lie between the mobile and immobile limits for film thickness range of 100–2000 nm thereby indicating that the effects of surface rheological properties on film stability is indeed important. Also, for very large film thickness (>2000 nm), the film can be considered to be mobile, whereas for very thin films (<100 nm), the film can be considered to be immobile.

15.4 Layer by Layer Electrostatic Deposition

15.4.1 Principle of Layer by Layer (LbL) Deposition

Conventional single layer emulsion, which is produced by mixing or homogenizing oil and aqueous phases together in the presence of emulsifier, may have limited stability under certain environmental conditions, such as pH, temperature, ionic strength, etc. (Guzey and McClements 2006). This problem can be overcome by employing a technique known as layer by layer deposition in which the emulsion droplets are coated with multilayers of emulsifiers (Guzey et al. 2004; Moreau et al. 2003; Ogawa et al. 2003).

In early 1990s, Decher et al. (Decher and Hong 1991a, b; Decher et al. 1992) introduced a method of creating multilayer films using oppositely charged polymers. This resulted in the revolutionary of LbL electrostatic deposition technique. This technique became popular for preparing polyelectrolyte capsules, because it is a simple, inexpensive and easily controllable method to create highly tailored capsule (Peyratout and Dähne 2004).

The principle of producing LbL deposition is simple and straightforward: sequential adsorption of oppositely charged materials on a template to form polyelectrolyte shells (Guzey and McClements 2006; Humblet-Hua et al. 2011; McClements 2005). The method of producing LbL deposition is demonstrated in Fig. 15.22. Firstly, charged surface active compounds were added into two immiscible liquids, the mixture was homogenized to form the primary emulsion. Then, a second solution containing oppositely charged polyelectrolytes were added into the primary emulsion and mixed, the oppositely charged polyelectrolytes would be absorbed onto the surface of emulsion droplets due to electrostatic interaction, and

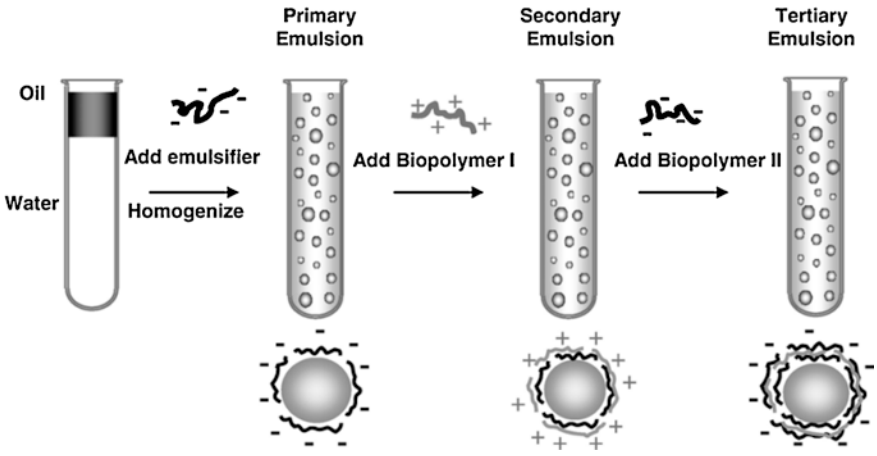

Fig. 15.22 Scheme of the buildup of LbL deposition. (Adapted from Guzey and McClements 2006)

this resulted in the formation of secondary emulsion. By repeating the addition of polyelectrolytes that carry opposite charges in each step, emulsion stabilized by multiple layer deposition can be produced. It is to be noted that between each step, the removal of excess free polyelectrolytes is critical; otherwise, the addition of oppositely charged polyelectrolytes in the next solution would interact with the free polyelectrolytes, which may interfere with the formation of the multilayers around the emulsion droplets (Guzey and McClements 2006).

15.4.2 Experimental Protocol and Characterization

The experimental protocol for producing LbL deposition involves (1) preparation of primary oil in water emulsion (either by mixing or homogenization) stabilized by charged polyelectrolyte in the aqueous phase, (2) separation of cream layer and removal of aqueous medium consisting of excess polyelectrolyte, (3) addition of oppositely charged second polyelectrolyte solution to the cream layer, (4) remixing to form secondary emulsion, (5) repeat steps (1) through (4) with third polyelectrolyte solution of charge opposite to that of second polyelectrolyte, etc. to make emulsion stabilized by multiple layer deposition.

An example of producing LbL deposition is given by Humblet-Hua et al. (2011). Ovalbumin (OVA) fibril (positively charged) and high methoxyl pectin (HMP, negatively charged) were used as the two oppositely charged polyelectrolytes that construct the shell wall. 0.05% wt OVA fibril solution was added to a 2% wt emulsion of n-hexadecane, and mixed with a rotor-stator mixer at 13,500 rpm for 90 s. Then the emulsion was subjected to 50 × g centrifugation for 45 min at 20 °C to remove the non-adsorbed OVA fibril. Next, the emulsion was centrifuged at 750 × g for 1 min to remove excess solution. After this step, the concentrated emulsion was dispersed into a HMP solution (0.05% wt) at pH 3.5. Since HMP is negatively charged at pH 3.5, it was adsorbed onto the surface of emulsion droplets, which was covered by positively charged OVA fibril. This bi-layer emulsion was isolated by centrifugation, and dispersed into OVA fibril solution to form the third layer. By repeating this process, emulsion droplets with multiple layers were produced.

Three common strategies that can be used to remove the excess free polyelectrolytes are saturation method, centrifugation method and filtration method (Guzey and McClements 2006; Voigt et al. 1999). In saturation method just adequate amount of polyelectrolytes are added to the solution in order to encapsulate the droplets. The amount of polyelectrolytes is determined by ζ potential measurement which is used to ensure that free polyelectrolytes in the solution are so few that they cannot result in bridging flocculation (Guzey and McClements 2006). In centrifugation method, on the other hand, more than enough polyelectrolytes are added to the aqueous phase, which are then are removed by centrifugation. The centrifugation separate droplets and aqueous phase which contains free polyelectrolytes. This centrifugation step is normally repeated several times so that most of the excess polyelectrolytes have been removed before the addition of next polyelectrolytes (Guzey and McClements 2006). In the case of filtration method, non-adsorbed polyelectrolytes are removed by membrane filtration. The filtration membrane induces a

pressure that forces the aqueous phase (along with free polyelectrolytes) to pass through the membrane so that the particles and aqueous phase containing free polyelectrolytes can be separated (Guzey and McClements 2006). It is important to choose one of these three methods to produce stable multilayer systems without promoting droplet aggregation.

Different analytical techniques were used to characterize the properties of LbL emulsion. The most essential properties for LbL emulsion that are of interest are the size distribution of the emulsion droplets, the net electrical charges on the surface of emulsion droplets, the thickness and composition of the shell wall, and the long term stability of the emulsion (Guzey and McClements 2006). The commonly used methods to measure droplet size of LbL emulsion include scattering methods, such as dynamic light scattering (DLS) (Okubo and Suda 2003; Sukhorukov et al. 1998), single particle light scattering (SPLS) (Caruso et al. 2000; Park et al. 2001; Schönhoff 2003), small-angle neutron scattering (SANS) (Estrela-Lopis et al. 2002), etc. DLS can also be used to calculate the zeta (ζ) potential, which reflects the sign and magnitude of the electrical charge on the emulsion droplet surface (Guzey and McClements 2006; Humblet-Hua et al. 2012). In addition, the amount of adsorbed electrolytes on the surface of emulsion droplets can be measured by Fourier Transform Infrared Spectroscopy (FTIR), which determines a linear scale of surface concentration of the adsorbed polyelectrolytes by providing integrated band area from the band positions assigned to infrared vibrations of the polyelectrolytes (Caruso et al. 1998b; Guzey and McClements 2006; Reihs et al. 2003). The size, shape, morphology and wall structure of the multilayer emulsion droplets can be examined by microscopy methods, which include scanning electron microscopy (SEM) (Caruso and Möhwald 1999a, b; Reihs et al. 2003; Sagis et al. 2008), transmission electron microscopy (TEM) (Caruso et al. 1998a; Kato et al. 2002; Sagis et al. 2008; Yang et al. 2001), atomic force microscopy (AFM) (Mermut et al. 2003) and confocal laser scanning microscopy (CLSM) (Caruso et al. 1999; Ibarz et al. 2002). Figure 15.23 shows images of oil-in-water emulsion droplets encapsulated

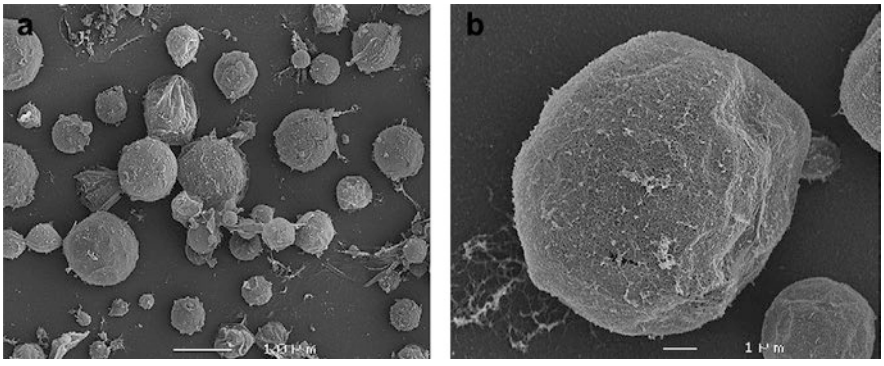

Fig. 15.23 Images of microcapsules encapsulated with four-layer ovalbumin fibrils and HMP under SEM, the inner phase (oil) was removed by critical point drying. (Adapted from Humblet-Hua et al. 2011) (**a**) low magnification (**b**) high magnification

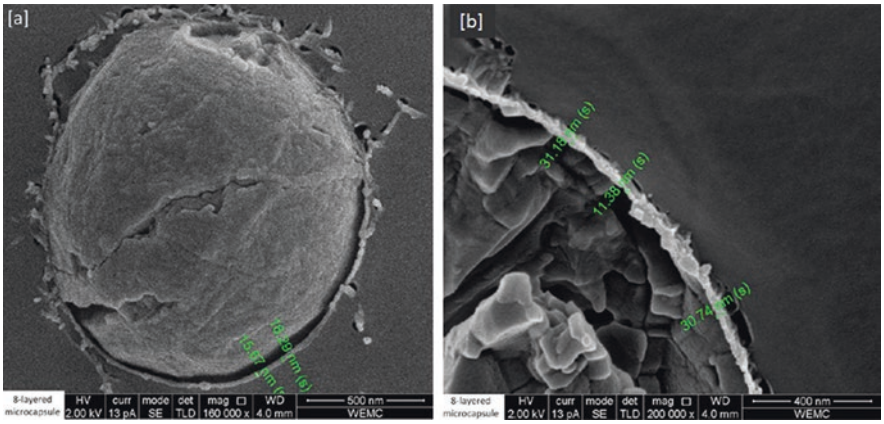

Fig. 15.24 Images of oil-in-water emulsion droplet encapsulated with 8-layer lysozyme fibrils and HMP under cryo-SEM. (Adapted from Humblet-Hua et al. 2012) (**a**) low magnification (**b**) high magnification

with four-layer ovalbumin fibrils and HMP under SEM (Humblet-Hua et al. 2011). The inner phase of microcapsules and thickness of the shell wall can be investigated by cryo-SEM, which connects a cryo system (a freezing unit, a turbo-pumped cryo preparation unit, and a cryo stage) with SEM. The emulsion droplets undergo flash freezing in liquid nitrogen slush followed by high vacuum sublimation of unbound water in the cryo system, followed by freeze-fracture so that the inner phase of the droplets are exposed. An example of freeze-fractured droplet covered by eight-layer lysozyme fibril and HMP is given in Fig. 15.24, with measured thickness of droplet shell wall (Humblet-Hua et al. 2012). CLSM can be used to determine the distribution of encapsulating materials on the emulsion droplets, by using different fluorophores or dyes to attach to the encapsulating materials (Humblet-Hua et al. 2011, 2012; Sagis et al. 2008).

The factors that affect stability of LbL deposition include concentration of droplet and polyelectrolytes, size of droplet, characteristics of polyelectrolytes and numbers of layer that encapsulate the droplets. According to Guzey and McClements (2006), both low and high concentration of polyelectrolytes may result in bridging flocculation, because at low polyelectrolyte concentration, there is inadequate polyelectrolyte to saturate the surface of all the droplets, on the other hand, at high polyelectrolyte concentration, the bridging flocculation can also occur since the polyelectrolytes may not be able to adsorb onto the surface of droplets to prevent flocculation. According to their calculation, for a stable LbL system consisting of droplets with radius of 0.3 μm and polyelectrolyte with molecular weight of 100 kDa and a radius of gyration of 30 nm, the droplet concentration should be as low as $\phi < 0.11$ (11 vol.%) with an intermediate polyelectrolyte concentration ($C_{Adsorption} < C < C_{Depletion}$). For the same system, if the droplet size has an average radius less than 0.15 μm, the system would not be stable even with an intermediate polyelectrolyte concentration, because polyelectrolytes would not be able to cover

the surface of the droplets before they collide with each other. The mechanical stability can be improved by increasing numbers of layer that encapsulate the emulsion droplets. According to Humblet-Hua et al. (2012), the heat resistance time (heating at 90 °C) increase from 2 s to more than 150 s when increasing the number of layers of lysozyme and HMP from 2 to 8. Furthermore, they also demonstrated that the characteristics of the encapsulation materials is also important by comparing ovalbumin-HMP and lysozyme-HMP complexes as coating material, and demonstrated that ovalbumin-HMP complexes can construct a more compact and stretchable shell and therefore, resulted in more stable emulsion (Humblet-Hua et al. 2012).

Lee et al. (2009) employed homogenization (2000 psi) as the first step to prepare the primary emulsion, before the addition of secondary layer; 40,000 × g centrifugation for 60 min was employed to separate the cream layer. Güzey and McClements (2006)) also reported using high pressure homogenization as the first step. However, in order to provide stable emulsion, the flocs that were formed by excess protein were disrupted by rehomogenization for two passes (2000 and 200 psi). Preventing bridging flocculation was necessary for the production of LbL emulsion. Using homogenization as the first step to produce desired small emulsion droplets requires a more difficult separation step to isolate the cream layer. In order to circumvent this, Xiang et al. (2016) investigated the employment of homogenization as the second step to produce stable LbL emulsion. In this study, they employed the above technique to produce fish oil in water emulsions encapsulated with a positively charged inner soy β-conglycinin (7S) layer by high shear mixing followed by deposition of a negatively charged outer high methoxyl pectin (HMP) layer using homogenization (500 or 3000 psi). The size, shape, morphology and wall structure of 7S and HMP encapsulated emulsion droplets were characterized.

The emulsions created by homogenization at 3000 psi had a proteinaceous interfacial layer capable of binding to thioflavin T (ThT), as evident from the staining of the rims of the emulsion drops in Fig. 15.25 (Xiang et al. 2016). The fluorescence signals confirm the presence of adsorbed protein layer on the emulsion droplets. Compared to three-layer microcapsules covered by whey protein isolates (WPI) and HMP (Sagis et al. 2008), 7S was found to be distributed more uniformly on the surface of oil droplets as evidenced by a stronger and less diffuse fluorescence signal around the rim, which indicates that 7S is more flexible and surface active than WPI (Xiang et al. 2016). When HMP was used as the second layer to cover the emulsion droplets, ThT fluorescence signal can still be detected, as shown in Fig. 15.25b, which indicates that there are gaps between the covered regions of HMP, so that ThT molecules can bind to exposed 7S on the surface of emulsion droplets thereby indicating that the outer HMP layer is porous (Rossier-Miranda et al. 2012; Xiang et al. 2016). Additionally, emulsion droplets that were obtained by layer by layer deposition showed a smaller size distribution, which indicated that the additional layer of HMP helped to prevent droplets from flocculation possibly due to steric interaction. The emulsion droplets that were produced by homogenization in the presence of 7S-HMP complex showed a more diffuse interfacial adsorbed layer as shown in Fig. 15.25c. The intensity distribution for the three cases as shown

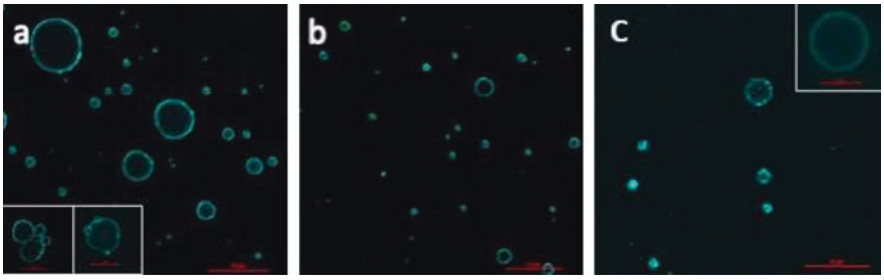

Fig. 15.25 Confocal laser scanning microscopy images of emulsion samples (3000 psi homogenized) stained by the thioflavin-T (ThT) dye and excited at 440 nm. Emissions were collected at 482 nm. (**a**) Emulsion droplets covered with 7S in pH 3 buffer. (**b**) Emulsion droplets with primary layer cover of 7S and second layer of HMP. (**c**) Emulsion droplets covered with 7S-HMP complex (Xiang et al. 2016)

in Fig. 15.26. Xiang et al. (2016) shows that the profile is sharpest for 7S stabilized emulsion and most diffuse for 7S-HMP complex stabilized emulsion. It is understandable that 7S is more diffuse in the adsorbed layer in case of 7S-HMP complex since it can be attached to HMP at any location along the length of the molecule. On the other hand, 7S is located predominantly in the inner layer in case of LbL thereby leading to a much smaller region of 7S. The discrepancy between the adsorbed 7S regions for pure 7S and LbL can be attributed to swelling behavior of multilayer shells (Schönhoff 2003). The thickness of 7S layer was less for LbL compared to 7S-HMP complex thereby indicating an inner compact layer for the former (Xiang et al. 2016). Moreover, ζ potential for LbL was more negative compared to 7S-HMP complex. The morphology of outer surface of the droplet was also found to be smoother (Xiang et al. 2016). These differences indicate that the structure of adsorbed layer in case of LbL is closer to that formed by an inner 7S layer and an outer HMP layer. The applicability of first high shear mixing step followed by homogenization as the second step for formation of emulsions using LbL was demonstrated by producing emulsions that exhibited superior stability compared to conventional high shear mixing in both steps (Xiang et al. 2016). In addition, these emulsions were stable for a few weeks.

15.4.3 Potential Applications in Food

Proteins, nanoparticles, dye molecules, and variety of other macromolecular species can be encapsulated into the polyelectrolyte films using LbL deposition technique (Decher 1997; Yang et al. 2001). Different types of emulsifiers can be applied to LbL deposition to improve the stability of the emulsion against environmental stress, such as pH extremes, high salt concentration, heating/freezing processing, dehydration and mechanical agitation (Guzey and McClements 2006).

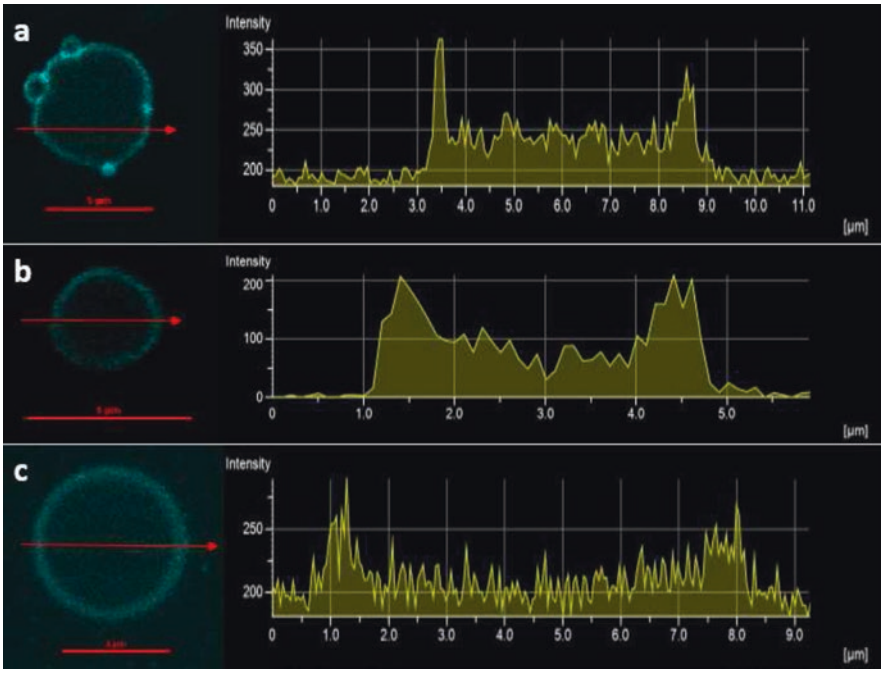

Fig. 15.26 Fluorescence intensity scan through a droplet along an arrow shown in red. (**a**) Emulsion droplets covered with 7S in pH 3 buffer. (**b**) Emulsion droplets with primary layer cover of 7S and second layer of HMP. (**c**) Emulsion droplets covered with 7S-HMP complex (Xiang et al. 2016)

In the production of food emulsions, the choice of encapsulation materials depends on whether the electrical charges of the encapsulation materials can provide enough electrostatic attraction between each layer, and whether the interfacial charges provide sufficient electrostatic repulsion to prevent the aggregation of the droplet. The pH of different food emulsion products can vary from acidic (pH 2.5–4) to slightly alkaline (pH 7–7.4) (Guzey and McClements 2006). In an acidic aqueous environment, pectin has been shown to exhibit strong adsorption to β-lactoglobulin-coated droplets at pH 3–5 (Guzey et al. 2004; Guzey and McClements 2006; Moreau et al. 2003). Additionally, fish gelatin can be used to adsorb onto the surface of SDS-coated droplets for a wide range of pH from 3 to 7 (Surh et al. 2005). A number of studies have demonstrated that emulsions produced by LbL deposition are more stable than single-layer emulsions especially at high ionic strength (Aoki et al. 2005; Gu et al. 2005; Guzey et al. 2004; Guzey and McClements 2006; Ogawa et al. 2003).

For instance, single-layer lecithin-coated droplets were unstable at $\geq$3 mM; However, lecithin–chitosan-coated droplets were stable at $\leq$500 mM $CaCl_2$ (Aoki et al. 2005). Thermal processing is a necessary step for many food emulsion production processes, however, many emulsifiers are not able to prevent aggregation during thermal processing.

For example, some small molecule surfactants have a phase inversion temperature (PIT) which results in coalescence during thermal processing (David et al.

1998). Multilayer emulsions coated by anionic surfactant and a cationic polyelectrolyte, on the other hand, have been shown to display high stability to thermal processing from 30 to 90 °C, such as SDS–chitosan (Aoki et al. 2005), lecithin–chitosan (Ogawa et al. 2003) and SDS–gelatin (Surh et al. 2005). For chilling and freezing, SDS–chitosan–pectin system can be used as an example of improving stability of emulsion to cold storage (−20 °C for 22 h) (Aoki et al. 2005). In order to convert oil-in-water emulsion to a powdered form, dehydration methods such as spray drying or freeze drying are often used in food industry (Guzey and McClements 2006; Ortega-Rivas et al. 2006). Comparison of the stability to freeze drying and spray drying between single-layer and multiple-layer emulsions were carried out by Klinkesorn et al. (2005a, b), who demonstrated that multiple-layer emulsions had a much better stability to droplet aggregation than single-layer emulsions. Multilayer emulsions also show better stability to lipid oxidation than single-layer emulsions, which therefore can be applied to the encapsulation of polyunsaturated fats (such as ω-3 fatty acids) into food products (Guzey and McClements 2006; Klinkesorn et al. 2005a; Ogawa et al. 2003).

More work needs to be done to demonstrate the applicability of LbL deposition technique to incorporate sensitive food components, such as enzymes, flavors, peptides, bioactive lipids, antioxidants and antimicrobials (Guzey and McClements 2006; Shahidi and Han 1993). The LbL deposition technique is a simple, inexpensive and easily controllable technique for production of food emulsion because of its ability to provide better stability to environmental stress, and therefore holds a great promise in food industry.

15.5 Microemulsion

15.5.1 Definition

Generally, droplet size of common emulsions (or, macroemulsion) is in the order of one micrometer, typically 0.2–10 μm. Such emulsions are turbid, kinetically stable but thermodynamically unstable. Microemulsions are defined as systems that are comprised of a mixture of water, hydrocarbons, and amphiphilic compounds which form thermodynamically stable, homogeneous (heterogeneous at molecular scale), optically isotropic solutions. Droplet size of microemulsions is usually in the range of 5–100 nm (Flanagan and Singh 2006).

15.5.2 Formation and Stabilization

15.5.2.1 Formation

Theoretically, there is a spontaneous arrangement of surfactants (or surfactants with cosurfactants) on the surface of microemulsion droplets. However, for some system, the input of energy can speed up the arrangement of surfactants on microemulsion

droplets, and help to overcome small kinetic energy barrier. According to Flanagan and Singh (2006), there are three methods that are commonly used to produce microemulsion:

15.5.2.1.1 Low Energy Emulsification Method

Microemulsions are formed spontaneously by diluting an oil-surfactant mixture with water, or diluting a water-surfactant mixture with oil, or mixing all the components together. The order of addition ingredients has an impact effect on the formation of microemulsion (Flanagan and Singh 2006).

15.5.2.1.2 Phase Inversion Temperature (PIT) Method

The PIT method is used when ethoxylated non-ionic surfactants are employed in the microemulsion system. Increasing temperature to a critical temperature (the PIT) would result in an o/w emulsion containing ethoxylated non-ionic surfactants o/w emulsion inverting to a w/o emulsion. The droplet size and interfacial tension would reach to a minimum at PIT. A stable o/w emulsion would be formed after cooling and stirring (Flanagan and Singh 2006).

15.5.2.1.3 High Pressure Homogenization

Microemulsion can also be formed by homogenization. However, due to the dissipation of heat, the process of emulsification is generally inefficient. Moreover, since the water/oil/surfactant mixture could be highly viscous prior to microemulsion formation, the homogenization process could be extremely limited (Flanagan and Singh 2006).

15.5.2.2 Thermodynamics of Formation of Microemulsions

Microemulsions are homogeneous mixtures of oil and water which are transparent. The emulsion drop sizes in microemulsions are very small, usually in the range of 5–50 nm. These drops are stabilized by large amounts of surfactants and cosurfactants with high salt concentrations. Microemulsions are thermodynamically stable, i.e. the free energy of formation of microemulsion by breaking up one phase (either oil or water) into the second phase is negative.

When one breaks up one phase into very small drops with a very large interfacial area, this results in an increase in interfacial free energy. In addition, formation of large number of droplets would result in an increase in entropy of mixing with a corresponding decrease in free energy. The net free energy of formation of emulsion ΔF is given by

$$\Delta F = \gamma dA + \Delta F_{mix} = \gamma dA - T\Delta S \tag{15.88}$$

For macroemulsion, the entropy of mixing is negligible since the drop sizes are much larger and hence the total number of drops is small. In contrast, for microemulsions, the entropy of mixing is not negligible. Consequently, the free energy of emulsion formation is negative if the interfacial tension is sufficiently low thereby making emulsion formation spontaneous. Therefore, the main features that distinguish microemulsions from emulsions are their transparency, low viscosity and stability.

Therefore, the key to forming stable microemulsion is the reduction of interfacial tension which is achieved by the use of high concentrations of surfactant and a cosurfactant. Cosurfactants are usually short chain hydrocarbon tail surfactant molecules since they can penetrate the oil phase better than longer chain molecules. Addition of surfactants usually results in a considerable reduction of interfacial tension at short times because of rapid surfactant adsorption at the interface. This is usually followed by an increase in interfacial tension which eventually reaches an equilibrium value. The latter behavior is due to depletion of surfactant in the bulk as a result of its rapid adsorption at short times. In case of system with surfactant and cosurfactant, their interaction with the oil phase results in negative values of interfacial tension at short times (see Fig. 15.27) followed by its recovery. Negative or

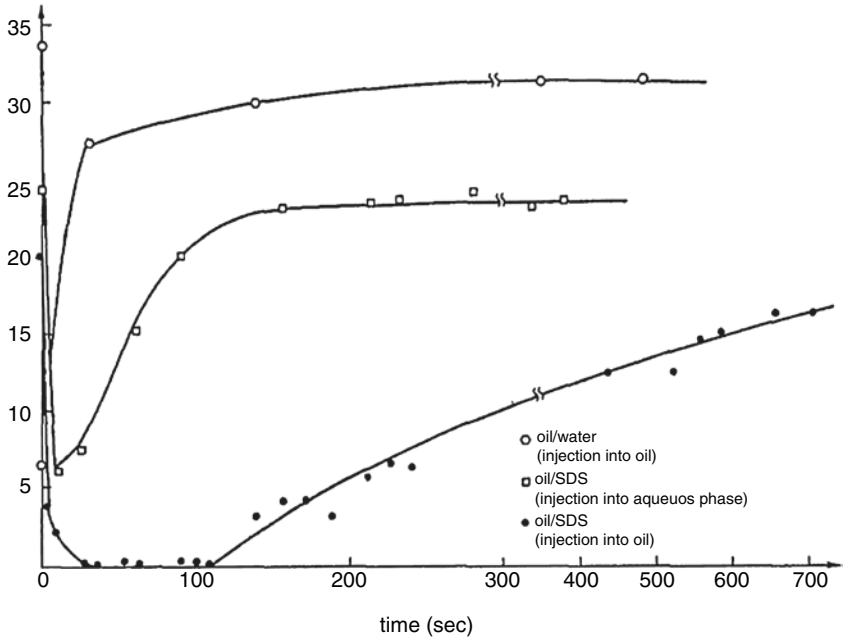

Fig. 15.27 Transient behavior of the interfacial tension in a typical microemulsion system (hexadecane water, SDS, pentanol). (Reproduced from Gerbacia and Rosano 1973)

very low values of interfacial tension results in spontaneous emulsification with high interfacial area leading to bicontinuous phase (Evans et al. 1986). When the interfacial tension is not very low, the dispersed phase exists in the form of spherical droplets since their deformation from spherical shape is resisted by Gibbs elasticity of the interface (Overbeek 1982). The curvature of the interface is governed by several factors, the most important of them being the size of hydrophilic or hydrophobic residues of the surfactant and cosurfactant. A smaller hydrophilic head with a shorter hydrocarbon tail will result in increased curvature (small drop size) of the interface. In addition, a water in oil microemulsion is favored for (1) a longer chain cosurfactant, (2) smaller size of head group, (3) branched chain in the surfactant, (4) higher salt concentration and (5) polyvalent counterion.

Prediction of choice of systems and conditions for formation of microemulsion would involve identification of conditions for the free energy of formation of microemulsion to be negative for a positive droplet size. Ruckenstein and Krishnan (1980b) investigated the adsorption of mixture of non-ionic surfactant and cosurfactant (with different areas per molecule) at the oil-water interface and derived the following expression for the interfacial tension γ as a function of surfactant and co-surfactant concentrations c_1 and c_2 respectively to give

$$\gamma = \gamma_0 - NkT \ln\left[1 + \left(K_1 c_1\right)^{1/p_1} + \left(K_2 c_2\right)^{1/p_2}\right] \tag{15.89}$$

where K_1 and K_2 are related to adsorption and desorption constants and p_1 and p_2 are the numbers of surface sites occupied by one molecule of surfactant and cosurfactant respectively. The free energy of formation of emulsion consisted of surface free energy (due to interfacial tension) and entropic contribution resulting from breaking up the oil phase in the form of a very large number of droplets. The surface free energy was evaluated using the above expression for interfacial tension which depended on the surfactant and co-surfactant concentrations. The free energy contribution due to entropy of mixing was evaluated by Ruckenstein and Krishnan (1980b) using lattice model (Ruckenstein and Chi 1975). The critical droplet size for microemulsion was then evaluated by minimizing the free energy and is given by,

$$R_e = \frac{3N\phi\left(E-1\right)\left(1-K_1^{-1}\nu_1\left(E-1\right)\right)}{p_1 E\left(n_1 - K_1^{-1}\left(1-\phi\right)\left(E-1\right)\right)} \tag{15.90}$$

where $E =$ exp ($\gamma_0 p_1/kTN$). From the above equation, one can see that $R_e > 0$ only if the amount of surfactant n_1 is greater than $K_1^{-1}\left(1-\phi\right)\left(E-1\right)$. Two conditions have to be satisfied for the formation of microemulsion, (1) minimum free energy of formation should be negative and (2) equilibrium drop size should be positive. The above expression gives a minimum surfactant concentration for the drop size to be positive. In addition, Ruckenstein and Krishnan (1980b) showed that in the absence of cosurfactant one cannot form stable microemulsion. In the absence of

cosurfactant, surfactant can form micelles at sufficiently high concentration thereby restricting its ability to reduce the interfacial tension as a result of dilution. Cosurfactant, on the other hand, can compensate for this by its adsorption at the interface thereby reducing the interfacial tension. Also, cosurfactant does not associate into micelles. In the presence of cosurfactant, the interfacial tension can decrease to extremely small value thereby promoting the formation of microemulsion as can be seen from Fig. 15.28.

In the case of ionic surfactants, the adsorption behavior is influenced by electrostatic effects and is therefore greatly influenced by ionic strength. Ruckenstein and Krishnan (1980a) expressed the electrical contribution of free energy of formation of microemulsion as

$$\Delta F_{el} = -\int \sigma d\psi_0 \tag{15.91}$$

Therefore,

$$\gamma = \gamma_0 + \Delta F_{ads} + \Delta F_{el} \tag{15.92}$$

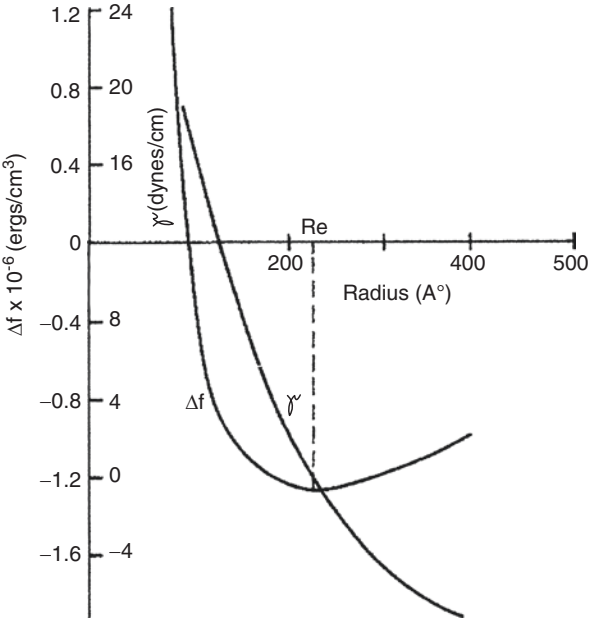

Fig. 15.28 Free energy of formation ΔF as a function of radius of microemulsion drops for a hypothetical system. Total quantity of surfactant and cosurfactant in the system is 0.1 mol/lit; $K_1 = 0.12 \times 10^{-24}$ m^3 molecule^{-1}; $p_1 = 2$; $p_2 = 1$. Molecular volumes: $v_1 = 10$, $v_2 = 3.5$, $v_{oil} = 4.87 \times 10^{-28}$ m^3 respectively. Volume fraction of dispersed phase is 0.1. Surfactant is confined to the continuous phase and cosurfactant is confined to oil phase. (Reproduced from Ruckenstein and Krishnan 1980b)

The contribution to free energy due to surfactant adsorption ΔF_{ads} is given by,

$$\Delta F_{ads} = -NkT \ln\left\{1+\left[K_1 c_R \exp\left(-e\psi / kT\right)^{1/p_1}\right]+\left(K_2 c_{cs}\right)^{1/p_2}\right\} \tag{15.93}$$

where c_R is the concentration of ionic surfactant, ψ is the potential and c_{cs} is the concentration of cosurfactant. As ionic strength increases, more ionic surfactant adsorbs onto the oil-water interface as a result of shielding of charges thereby making ΔF_{ads} more negative. Eventhough this results in higher surface charge density σ, ΔF_{el} as given by equation does not increase but reaches a minimum value because the potential ψ decreases because of shielding of charges. Compared to nonionic surfactant, ionic surfactant is not able to decrease the interfacial tension as effectively because of the charge repulsion preventing its adsorption at high concentration. As a result, presence of cosurfactant is even more essential in this case to further decrease the interfacial tension. It is to be noted that adsorption of cosurfactant is not impeded by electrical effects since it is nonionic.

Overbeek (1982) expressed the free energy contribution to entropy of mixing in terms of Carnhan-Sterling approximation for the osmotic pressure of a collection of hard spheres and obtained the following expression

$$\Delta F_{mix} = \frac{A^2 kT}{12\pi\left(n_w v_w\right)^2}\left(\ln\phi - 1 + \frac{\phi\left(4-3\phi\right)}{\left(1-\phi\right)^2} + \ln\frac{v_o}{v_{hs}}\right) \tag{15.94}$$

where n_w is the number of moles of water, v_w is the molar volume of water, v_o is the molar volume of oil, v_{hs} is the molar volume of hard spheres and ϕ is the volume fraction of oil. For charged systems, the effective hard sphere volume (accounting for the presence of double layer) should be used.

As pointed out above, whether an O/W microemulsion or W/O microemulsion will be formed depends on the relative sizes of hydrophilic head group and hydrocarbon tail, i.e. the geometric factor. Israelachvili et al. (1976) have applied the analysis of relationship of packing geometry to micellar structure to microemulsions. Schematic of O/W and W/O emulsion droplet with surfactant is shown in Fig. 15.29. From geometric considerations, one obtains (Israelachvili et al. 1976)

$$x = \left(\frac{3v}{A_0 l_c} - \frac{3}{4}\right)^{1/2} - \frac{1}{2} \tag{15.95}$$

where v is volume occupied by each hydrocarbon chain, A_0 is the area occupied by a hydrocarbon head and $x = r_o/r_w$. Consequently, for an O/W microemulsion, $x < 1$ which implies that $v/A_0 l_c < 1$. Similarly, $x > 1$, i.e. $v/A_0 l_c > 1$ for W/O microemulsion.

Based on these, Oakenfull (1980) has derived the following expression for the maximum droplet size

$$r_{\max} = \frac{l_c^2}{\left(\frac{v}{A_0} - l_c\right)} \tag{15.96}$$

For a microemulsion, $r_{\max} > 0$. Consequently, $v/l_c > A_0$. For a typical value of $A_0 = 0.66$ nm^2, $v/l_c > 0.7$ nm^2. In cases where the condition cannot be satisfied by a single surfactant because of its large hydrophilic head area, microemulsion cannot be formed. Addition of small molecule cosurfactant will satisfy this condition because of smaller overall hydrophilic area (more efficient packing of cosurfactant) thereby promoting the formation of microemulsion.

15.5.2.3 Phase Behavior of Oil-Water-Surfactant Systems

In order to formulate a three (oil, water, surfactant) or four component (oil, water, surfactant, cosurfactant) microemulsion system with as little surfactant and/or cosurfactant, it is desirable to identify concentration region within which different components are miscible. This would require the knowledge of phase behavior of such systems. In the following, we will briefly discuss the salient aspects of phase behavior of such systems. Typical phase diagram of a three component system of oil, water and amphiphile is shown in Fig. 15.30. At low concentrations of surfactant, oil and water are not miscible as indicated by the two phase region whose compositions are described by tie lines. As the surfactant concentration is increased,

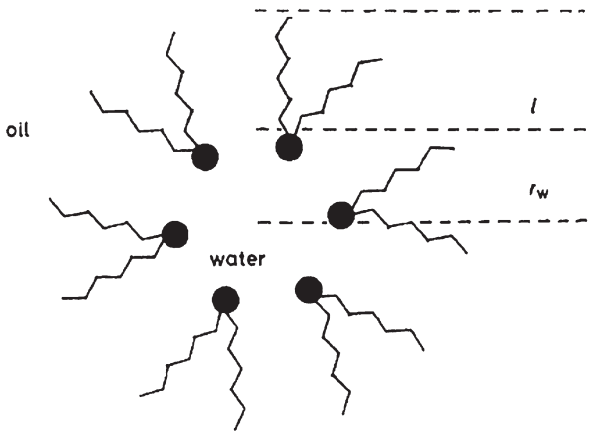

Fig. 15.29 A microemulsion droplet (Oakenfull 1980)

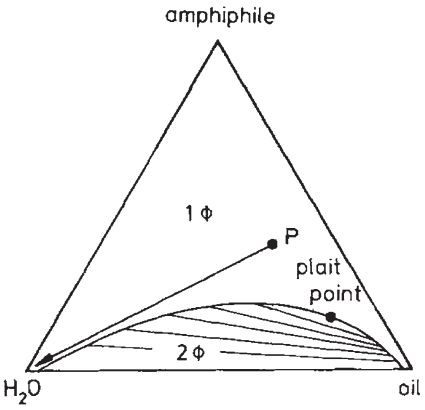

Fig. 15.30 Typical ternary diagram of water, oil and surfactant mixture. Tie lines and plait point in the two phase region are shown. Single phase region is indicated by 1ϕ. (Reproduced from Kahlweit and Strey 1985)

the tie lines become shorter eventually shrinking into a single point known as plait point. At surfactant concentrations above this critical value, the three components are completely miscible.

The phase diagram for such a system has been classified into three classes by Winsor (1948).

I two phases with amphiphile dissolved mainly in aqueous phase.
II two phases with amphiphile dissolved mainly in oil phases.
III three phase.

The phase behavior is sensitive to variations in temperature. At low temperatures, the system can be described as class I. At high temperatures, it behaves like class II and at intermediate temperatures it can be classified as class III. The effect of temperature on phase behavior can be described by constructing phase diagrams over the three binary sides of the ternary diagram with temperature as the vertical coordinate (see Fig. 15.31).

The phase diagram of oil water mixture at different temperatures show very large immiscibility region. The miscibility region increases slightly with temperature. Usually, the upper critical solution temperature is found to be greater than the boiling points of both components. The binary mixture of oil and non ionic surfactant are mostly immiscible at very low temperatures. The upper critical solution temperature is around 0 °C. On the other hand, the behavior of water-surfactant mixture is complex. They are mostly immiscible at low temperatures and become miscible at room temperatures. It exhibits a lower and upper critical solution temperatures especially at high surfactant concentration. At any temperature, the mixture forms association colloids (micelles) above a critical concentration. At higher concentration, it forms lyotropic mesophases (liquid crystals). The upper immiscibility region of water-surfactant mixture can be shrunk by increasing HLB value of the surfactant (i.e. increasing its hydrophilicity). The same effect can be achieved by adding hydrotopic salts (salts of either hard acid-soft base or soft acid-hard base) such as sodium alkyl sulphate. Conversely, addition of lyotropic salts (salts of hard

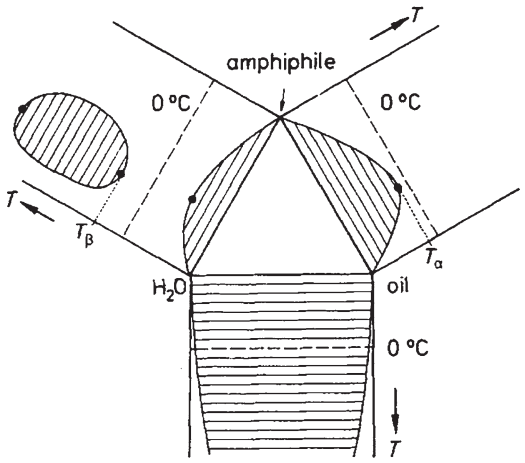

Fig. 15.31 Unfolded phase prism of the ternary system water, oil and amphiphile with phase diagram of three binary systems at different temperatures. (Reproduced from Kahlweit and Strey 1985)

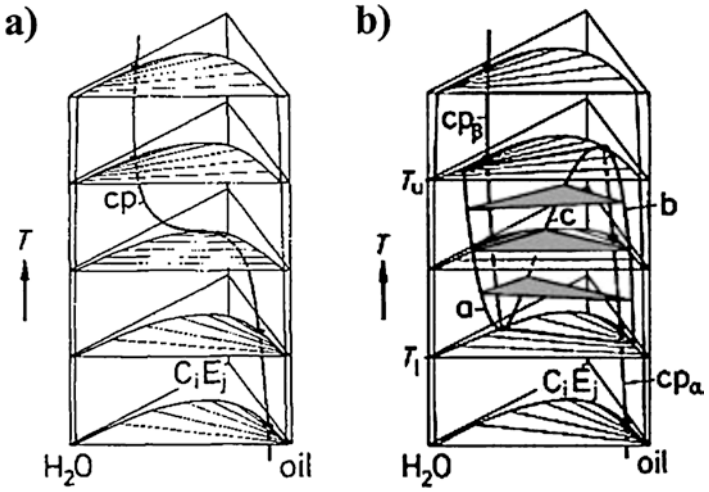

Fig. 15.32 Superposition of ternary diagrams at different temperatures. The critical line shown in both figures is the line joining the plait point of oil-water immiscibility region at different temperatures (**a**) type II system. Note the plait point going from oil rich to water rich regions as you go from low to high temperature. (**b**) Type III system. There is phase separation in the temperature range of T_l to T_u. (Reproduced from Kahlweit and Strey 1985)

acid-hard base)would expand the upper two phase region. When there is no immiscibility region for water-surfactant mixtures over the temperature range of interest, the three component system behaves like a type I system. On the other hand, when there is immiscibility region for water-surfactant mixtures as pointed out above, the system behaves like a type II system. In this case, the line joining the plait points at different temperatures (see Fig. 15.32a), goes from oil rich at lower temperature to water rich at higher temperatures.

For type III systems, there is phase separation in oil-water mixture between lower critical solution temperature T_l and upper critical solution temperature T_u. This can be clearly seen in Fig. 15.32b. Detailed description of phase behavior of quaternary system consisting of fourth component is given by (Kahlweit and Strey 1985)

15.5.3 Ingredients

Similar to common emulsions, microemulsions are composed of emulsifier, oil, and water. Generally, microemulsions also need cosurfactant.

Emulsifier, or usually surfactant, is critical to reduce interfacial tension of water-inter interface. At lower concentration, the surfactant exists as monomers, while above a certain minimum concentration, the surfactant molecules spontaneously associate to form micelles. This concentration is termed as critical micelle concentration (CMC). Surfactant concentration in microemulsions is very high, usually 20% or above.

Co-surfactants are used to further reduce interfacial tension. In addition, they may also increase interface fluidity. Commonly used cosurfactants are medium chain length alcohols, and amines and acids are also used in some cases.

Microemulsions are used as delivery systems for ingredients no soluble in water, therefore oil is a carrier. Usually hydrocarbon mineral oils are favorable for microemulsion formation. In contrast, high molecular weight oil, such as triglycerides containing long-chain fatty acids, is difficult to be used in microemulsion.

The formulation of food microemulsion is limited because of the consideration of toxicity issue. Double chain surfactants, for example sulfosuccinates, can form microemulsion without the presence of cosurfactants, however, these double chain surfactants are toxic in use of food product. Another problem that should be considered is that there are differences between food and other microemulsion in the composition of oil component. The oil in food, in most cases, is triglyceride, whereas in other microemulsion the oil is mostly a hydrocarbon (mineral oil). The triglyceride molecule itself is surface active, therefore, triglyceride is not able to form a separate oil phase unlike mineral oils (Paul and Moulik 2001). The difference in oil composition further limits the choice of surfactants. The currently used surfactants in food microemulsion include phosphatidycholine (lecithin), aerosol OT (AOT), sorbitan monostearate/monolaurate (Tweens), sorbitan mono-oleate, sorbitan monostearate, propylene glycol, etc. (Paul and Moulik 2001).

15.5.4 Applications in Food

During the past decades, functional foods have received more and more attention. Fortification of food with bioactive ingredients is one of the main approaches to make functional food. However, lots of such ingredients, such as β-carotene, lutein, fatty acids, flavonoids, probiotics, plant stanols, and phytoestrogens, etc., are

difficult to incorporate in food formulations because of their poor solubility in water. Microemulsion system is a potential way to solubilize them and easily incorporate them into regular food. Conventional emulsions have a particle size larger than 100 nm, therefore they appear opaque or turbid. Unlike emulsion, microemulsion has a particle size smaller than 50 nm and often appear transparent, therefore, its most important application is to produce foods or beverages that need to remain transparent (Rao and McClements 2011). In spite of promising application in food industry, there are still few reports and literatures on application of microemulsion to food products (Paul and Moulik 2001). The applications is limited because first, the lack of food-grade surfactants for preparing this system (Kralova and Sjöblom 2009). Second, it is difficult to prepare microemulsions with commonly edible oils, such as corn oil, fish oil or soybean oil, since it is difficult to incorporate large lipophilic molecules in microemulsion system (Salager et al. 2005). Nonetheless, some food-grade microemulsions are being developed. For example, Spernath et al. (2002) reported water-dilutable food-grade microemulsion consisting of ethoxylated sorbitan esters, which can be used as vehicles for delivering natural food supplements with nutritional and health benefits with enhanced solubilization (up to 10 times). Another important usage of microemulsion is that it can be used to improve the oxidative stability of certain oils. Soybean oil is effectively protected by using food-grade ethoxylated mono- and diglycerides (EMD) and phospholipids as surfactants with the presence of a short-chain alcohol (Flanagan et al. 2006). The oxidation of fish oil can be decreased if the oil is transformed into a microemulsion with ascorbic acid and α-tocopherol used as antioxidants (Jakobsson and Sivik 1994). Zhong et al. (2009) reported that microemulsion that consist of two surfactants, AOT [sodium bis(2-ethylhexyl) sulfosuccinate] and CrEL [Cremophor EL—polyoxyl (35) castor oil] in one to one ratio, can provide maximum protection of the flavor compounds in mint oil against degradation. Microemulsions of carnauba wax can also be utilized to coat fruits to minimize weight loss and internal oxidation. The fruits coated with microemulsions of carnauba wax show better appearance after washing and drying than other coatings, such as shellac, wood resin, oxidized polyoxyethylene or mixtures of these substances with carnauba wax (Paul and Moulik 2001). Rao and McClements (2011) reported using sugar monopalmitate as surfactant to stabilize the microemulsion which employ lemon oil as the oil phase, and examined the stability of sugar monopalmitate stabilized microemulsion against different environmental conditions.

15.6 Example of Emulsion Based Food Products

Emulsion exists extensively in food. The first food people eat upon birth, mammalian milk, is an emulsion. Homogenized milk with high pressure valve homogenizer was introduced in 1900 (Dickinson 1992). Nowadays, many food mixtures were made into emulsions to improve mouthfeel, texture, palatability, shelf life, and appearance. Becher (1985) summarized typical food emulsions with brief descriptions. In the following most important food emulsions are discussed.

15.6.1 Mayonnaise and Salad Dressing

Mayonnaise is a typical oil-in-water emulsion with high oil content. Corran (Becher 2001) has given a remarkably complete discussion about production of mayonnaise. The typical formula for a commercial mayonnaise is given in Table 15.4.

For a commercial product, flavoring and/or coloring materials are also added. Among the above ingredients, egg yolk is most critical for the stability of product. However, egg yolk is not a satisfactory emulsifier. The surface active components, lecithin and cholesterol, are only 11.5% of total weight. Lecithin is a good oil-in-water emulsifier while cholesterol is an effective water-in-oil emulsifier. If the lecithin/cholesterol ratio is low, e.g. 8:1 for a 50–50 oil-water emulsion, the emulsion may be inversed to water-in-oil. In natural egg yolk, the lecithin/cholesterol ratio is around 6.7:1. Therefore, mustard, which is a fine solid, is added to stabilize the mixture (Becher 2001). Other factors, such as phase volume, mixing method, water quality, and viscosity also have influence on the product (Becher 2001).

Salad dressing is another emulsion stabilized by egg yolk. The most difference from mayonnaise is the much lower oil content, which is usually around 45%. Additional stabilizer such as gums may also in presence. Compared to mayonnaise, stable emulsions of salad dressing is easier to obtain by any technique (Becher 2001).

15.6.2 Margarine and Table Spreads

Margarine was invented in 1869 as a butter substitute (Andersen and Williams 1965; Dickinson 1992). It is a water-in-oil emulsion with high content of oil. FDA standards of identity require the fat content no less than 80%. The water phase consists of water, salt thickeners, *etc*. The oil phase consists of partially hydrogenated vegetable oil, or sometimes animal fat (Borwanker and Buliga 1990). In modern times, marine oils, in particular oils from whale, are also used (Becher 2001).

Detailed information about margarine and table spreads is discussed in Chap. 13.

Table 15.4 Typical formula of commercial mayonnaise (Becher 2001)

Ingredient	Percentage
Oil	75.0
Salt	1.5
Egg yolk	8.0
Mustard	1.0
Water	3.5
Vinegar (6% acetic acid)	11.0

15.6.3 Beverages

Beverage emulsions are different from most of food emulsions in that the dispersed phase fraction is very small. The dispersed phase is the vehicle to carry flavors, colors and other oil-soluble ingredients (Becher 1985). This type of emulsions is difficult to prevent creaming because of the density difference between the dispersed and continuous phase. Viscosity of continuous phase and droplet size distribution also have influence on creaming (Chilton and Laws 1980).

15.6.4 Non-dairy Coffee Creamer

Creamers are typically applied to coffee or other beverages. Traditionally creamers are made with dairy products like whole milk, butterfat, or heavy cream, which all contains lactose. Although it is called non-dairy cream, it may contain sodium caseinate from milk. Non-dairy creamer is needed by the food market due to several reasons: (1) Traditional creamer contains lactose, which is not tolerated by many people; (2) Traditional creamer contains high level of saturated fat; (3) Some people do not eat dairy ingredients due to religious or personal beliefs; (4) Price of dairy products keeps increasing.

Typical non-dairy creamer is composed of protein, small molecular emulsifiers, vegetable oil, carbohydrate (usually corn syrup), minerals and flavors. In addition to soy protein, other vegetable protein may also be used, such as soy protein especially hydrolyzed soy protein, wheat protein, and others.

Typical process to make non-dairy creamer involves hydrating ingredients in water with mixer, followed by high pressure valve homogenization and sterilization. Sterilization is usually achieved by ultra-high temperature heating. The sterilized creamer may be packaged as liquid form, or more commonly, spray dried powder.

15.7 Guidelines for Selection of Food Emulsifiers

15.7.1 Regulatory of Emulsifiers

Emulsifiers are regulated by FDA in United States. Two groups of emulsifiers are classified: GRAS (generally recognized as safe) and Regulated Direct Food Additives. The former may be used in any non-standardized food product at any level necessary to obtain the desired technical effects. The latter may be regulated similarly to GRAS, but more often, they are strictly regulated in use, such as the methods of manufacture, analytical constants, type of food in which they are used, and maximum concentration.

15.7.2 Classification of Emulsifiers

There are many types of surfactants available to stabilize emulsions. Some classifications of surfactants has been developed based on the physico-chemical properties, such as Bancroft's rule, HLB number, and molecular geometry (Bergenstahl 1997; Davis 1994; Dickinson and Hong 1995). Among them HLB number is most extensively used.

HLB (Hydrophile-lipophile balance) is an empirical scale based on the relative percentage of hydrophilic and hydrophobic functional groups in the surfactant molecule (Griffin 1946). Surfactants with HLB numbers in the range 4–6 are suitable for stabilizing water-in-oil emulsions, whereas those with HLB numbers in the range of 8–18 are suitable for oil-in-water emulsions. HLB values of some commonly used food emulsifiers are given in Table 15.5. A group contribution technique (Davies 1957) for evaluating HLB of surfactant molecules assigns group numbers to different functional groups in the following equation,

$$HLB = 7 + \sum_i n_H(i) - \sum_j n_L(j) \tag{15.97}$$

where $n_H(i)$ and $n_L(j)$ are the group numbers of hydrophilic group i and hydrophobic group j, respectively. The group numbers of different functional groups are given in Table 15.6 (Davies 1957).

HLB concept does not account for the fact that functional properties of a surfactants strongly depends on temperature and solution conditions (Davies 1957). In reality, some surfactants are able to stabilize oil-in-water emulsions at one temperature while they stabilize water-in-water emulsions at other temperatures.

Table 15.5 HLB values for some food emulsifiers

Emulsifier	HLB
Sorbitan tristearate (Span 65)	2.1
Glycerol Monostearate	3.8
Sorbitan monooleate (Span 80)	4.3
Propylene glycol monolaurate	4.5
Succinic acid ester of monoglycerides	5.3
Sorbitan monopalmitate (Apan 40)	6.7
Sorbitan monolaurate (Span 20)	8.6
Diacetyl tartaric acid ester of monoglycerides	9.2
Polyoxyethylene sorbitan monostearate (Tween 60)	14.9
Polyoxyethylene sorbitan monopalmitate (Tween 40)	15.6
Polyoxyethylene sorbitan monolaurate (Tween 20)	16.7
Sodium oleate	18.0
Sodium steroyl-2-lactylate	21.0

Table 15.6 Hydrophilic group numbers

Group	n_H	Group	n_H
–SO_4Na	38.7	–COOH	2.1
–COOK	21.1	–H(free)	1.9
–COONa	19.1	–O–	1.3
Tertiary amine	9.4	–OH	0.5
Ester(sorbitan)	6.8	–(CH_2–CH_2–CH_2–O)–	0.33
Ester(free)	2.4	–(CH_2–CH_2–CH_2–CH_2–O)–	−0.15[a]

[a]The negative value denotes the group is lipophilic

In the food industry, proteins are also used as emulsifiers. Milk proteins, because of their high surface activity, are most extensively used. The two main classes of milk proteins are the caseins and whey proteins. β-Casein and α_{s1}-casein are the most important components of casein proteins. β-Casein is a flexible linear amphiphilic polyelectrolyte with a molecular weight of 24 kDa. At neutral pH, a β-casein molecule carries a net charge of −15e. It has little ordered secondary structure and no intramolecular covalent crosslinks. The hydrophobic and hydrophilic residues are non-uniformly distributed, which gives the molecule amphiphilic structure like a water soluble surfactant (Dickinson 2001). α_{s1}-Casein has a slightly smaller molecular weight but much higher net charge (−22e) at neutral pH. Its hydrophilic and hydrophobic residues are more randomly distributed (Dickinson and Matsumura 1994). Whey protein consists of several globular proteins, such as β-lactoglobulin and α-lactalbumin. β-Lactoglobulin has molecular weight of about 18.4 kDa from 162 amino acid residues. There are 5 cysteine residue with 2 intramolecular disulfide bonds and 1 free sulfhydryl group. At neutral pH, the net charge is −15e (Cornec 1999). In native state, the molecule is folded intramolecularly so that most of its hydrophobic residues are buried with the globular structure. The structure of β-lactoglobulin strongly depends on pH and temperature. It forms dimers at pH 7, while exists as monomers below pH 3.5 or above pH 7. Heating also changes the structure. When the heating temperature is lower at which the disulfide bonds are intact, the molecule may unfold and refold reversibly. However, when the heating temperature is high, the denaturation happens and the molecules become more disordered (Swaisgood 1996).

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Chapter 16
Future Trends of Emulsifiers and Other Food Ingredients

Gerard L. Hasenhuettl

16.1 Extrapolating the Line

Forecasting the future can be a risky proposition. Just ask anyone who has lost a significant amount of their wealth in the stocks or commodities markets. The simplest way to predict the future is to observe past trends, and assume they will continue. Of course real life throws discontinuities into the picture. Nutritionists are continuously discovering new nutrient behaviors, as well as old components that are detrimental to our health. Saturated fats were associated with cardiovascular disease, so scientist's developed Trans fats to replace them. Unfortunately, Trans fats simultaneously raise LDL and reduce HDL cholesterol. Now it appears that sugars and starches may be a greater health threat than fat. Many oils are derived from commercially grown crops, such as soybeans, coconuts, and palm fruits. Weather, pests, and disease can lead to dramatic crop losses, driving up the cost of the oil products.

A more thorough analysis of the future examines trends, such as aggregate income, income disparity, and consumer tastes and attitudes. As some of these trends approach a maximum or minimum value, there is a finite probability of a discontinuity in one or more trends. It is also illuminating to recognize interactions between trends.

Food emulsifiers and other ingredients are technologies that allow food scientists and product developers to respond to changes in trends. For example, when consumers decided that they preferred moist cakes, emulsifier blends were invented to retain moisture, without adversely affecting other desirable properties.

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16.2 Evaluating Consumer Diversity

Marketing professionals have long been familiar with diversity in human populations, and have skillfully segmented them into targeted markets. Targeting affluent consumers was considered a sure method for increasing gross margins, and therefore net income. However, during the recent recession, dollar stores began to target lower-income consumers. Consumer attitudes toward food additives are also trending. Jim Cramer recently noted on CNBC that consumers were divided into two groups. Consumers with adequate income were concerned about the long term effects of chemical additives. Poverty-stricken people were not concerned about long term effects, but were more anxious about surviving in the short term.

16.2.1 Consumers with Adequate Disposable Income

Affluent consumers are generally concerned about the long term effects of food additives, particularly genetically modified organisms, preservatives, and additives with chemical-sounding names. Several food emulsifiers fall into the latter category.

The central problem for formulators is to replace functionality of chemically produced surfactants with natural ingredients. There are three potential approaches. One is to use ingredients with foods that have surface and interfacial activity. Lecithin, milk fractions, and eggs will work in some applications, but may introduce other problems, such as microbial susceptibility. The second approach is to produce the chemically modified surfactants by using natural methods. Monoacylglycerols could be prepared by cleaving fatty acids with enzymes. This approach would almost certainly raise the cost. However, affluent consumers have some tolerance to absorb higher prices. A third possibility is to apply naturally occurring surfactants that are used in other industries. This would probably require more safety studies to obtain FDA approval, since there may not be a history of safe consumption.

16.2.2 Consumers with Inadequate Income

Consumers with inadequate income, to avoid hunger might like to avoid chemically produced food additives, but are unable to afford the increased cost. These consumers are increasing their intake of chemical surfactants, particularly in underdeveloped countries. The concern of formulators for these consumers is to insure that these surfactants are safe and have minimal color and off-flavors. There are fewer surfactant manufacturers in the United States because there are strict environmental regulations and low margins on commodity ingredients. Blending specialists can buy ingredients overseas and customize blends to meet customers' needs. Compliance with specifications is important when importing ingredients.

16.3 Nutrition for All Stages of Life

Food and water are essential for survival. Nutritional needs change as we progress through the human life cycle. Commercial production of food products dependent on what people need and want. Food emulsifiers play a key role in the palatability of these products, mainly because of their effects on texture and flavor.

16.3.1 Infant and Child Development

Prenatal nutrition is important to provide the building blocks for the developing fetus. Infants have no choice on what they are fed. Chapter 8 has a more detailed description of this area. As Toddlers begin to assert preferences, first as refusal, and then demands for certain foods. Although the science of early nutrition continues to develop (Nitzke et al. 2014), preference research for this age range is sparse or proprietary. Food emulsifiers and other additives can offer the possibility of making science-based diets more appetizing to the growing child.

16.3.2 The Prime Adult Years

Once children complete their education, leave home, and establish independent living, they become adults, capable of deciding their own diets. There preferences for foods become influenced by health, convenience, and peers.

Activity and nutrition interact to affect fitness, weight, intellect, and mood. Research on nutrition and mental performance is an emerging field (Riby et al. 2012). It is a brave new world where meal planning, learning, and brain imaging intersect. Nutrition and performance in competitive sports has been around longer and is gradually progressing (Jeukendurp and Gleeson 2009). Food and supplement producers have developed specialty products to optimize performance in speed, strength, and endurance.

Gender-specific nutrition has also emerged for sports and general health purposes (Hornstein and Schwein 2012; Matthews 2015a, b). Differences in muscle mass and structure, reproductive biology, and epigenetic disease resistance are driving research and product development. Both micro and macronutrients, as well as their interactions, are important.

Over the past century, life expectancy has increased dramatically in developed countries. Concern for the quality of life has led to research in geriatric nutrition (Morley and Colberg 2007; Hollins et al. 2013). Extensive research has also been conducted to extend the life expectancy of pets (Fascetti and Delaney 2012; Dodds and Laverdure 2015). As with human food products, pet foods have to have appealing flavor, texture, and smell. The products must appeal to the owners, as well as the pets. Pet foods have a different set of regulations governing additives, including emulsifiers (Federal Register 2013).

16.3.3 Individualized Nutrition

Dramatic progress in interpreting DNA structure, and more importantly relating it to physiology, has led to progress in a number of areas. DNA influences susceptibility to disease, development, and overall performance. We can now ask the question: does DNA determine which dietary regimen is best for a specific individual? The first attempts to answer the question may have been in a best-selling book that related optimal diets and exercises to blood type (D'Adamo and Whitney 1996). Blood type may serve as a crude approximation of DNA profile. More recent research has begun to entangle the gene structure and the chemical mechanisms which deal with food metabolism (Kaput and Rodriguez 2006; Kohlmeier 2012).

In addition to genetics, there are other variables between the plate and the bloodstream. Digestion efficiency depends on food structure, composition, type of microorganisms in the gut, and the kinetics of the digestive process (Boland et al. 2014). Absorption of nutrients also depends on the fat content and structure of excipient emulsions (McClements et al. 2015). The primary application of emulsifiers is to produce structured foods that have acceptable texture and flavor. At the present time customized food structures for each individual are impractical. Scientists and formulators can however begin to apply their knowledge to designing products for specific groups.

16.4 Sustainability

The United Nations estimates that the world's population will grow from 7.3 to 9.7 billion by 2050 and to 11.2 billion by 2100 (United Nations 2015). During the period 2015–2050, the largest population growth will be in nine countries. India is expected to surpass China to have the largest population by 2022, and Nigeria could pass the United States by 2050.

United Nations plans call for reduction in consumption of fossil fuels, efficient agricultural practices, and use of renewable sources for fuels and foods. Research is being directed to use of biomass to produce food ingredients from sources such as leaves and grass (Evans et al. 2014). A similar search for naturally surface-active molecules would also be logical.

Energy conservation could also be a driving force for further development of enzymatic processes. The problems of higher cost and difficult separations will be solved if energy costs drastically rise. Of course, the higher costs are likely to be passed along to consumers, many of whom are unable to afford them.

16.5 Future Challenges

The packaged food industry is an advertising-intensive, rather than an R&D-intensive business. The challenges of producing personalized, healthy, ecologically friendly, and desirably flavored products will require a broad scientific knowledge

base. The new products will therefore need a higher level of R&D spending than the industry budgets. The costs to consumers will initially be high, until innovation reduces them.

Increased scrutiny of food safety raises problems with a globalized supply chain. International trading rules may be harmonized along the lines of the FDA, EEC, and WHO standards. Food emulsifiers currently recognized as safe by the most nations will probably be accepted worldwide.

Consolidation in the food industry has resulted in fewer enormous global companies. R&D professionals will need to integrate developing technologies across a greater variety of food products.

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