9

Chemistry and Technology of Butter and Milk Fat Spreads

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9.1. Introduction

9.1.1. Legislation

Butter, margarine and spreads are viscoelastic solids (i.e., plastic emulsions that remain solid at room temperature and are mainly of the water-in-oil type). EU regulation (EC) No. 2991/94, in force since January 1996, provides definitions of spreadable fat products in the European Union. Butter, margarine and (full-fat) blends must contain not less than 80% fat but less than 90% fat, a maximum of 16% water and 2% non-fat milk solids. The salt content must be indicated on the label. Reduced-fat or three-quarter fat products should contain more than 41% but not more than 62% fat. Low-fat, light or half-fat products should contain 10–41% fat. Margarine should not contain more than 3% milk fat. Blended milk fat-vegetable fat products should have a milk fat content of 10–80% of the total fat. The milk fat may be modified only by physical processes, which in effect permits the use of thermally fractionated milk fat. It is also possible to produce zero-calorie fat spreads in which either fat substitutes or enzyme-resistant zero-calorie lipid-like materials, such as sucrose polyesters, are used (Mattson *et al.*, 1971). The development of low-fat spreads was first stimulated in the US by fat shortages during World War II. Oil-in-water spreads were the first to appear, in the 1950s and 1960s, but they had a number of technical problems. Water-in-oil spreads were first reported by Bullock (1966) but they did not gain a significant share of the edible fats market in the US (Behrens, 1988).

9.1.2. Emulsion Stability

Oil-in-water emulsion products, such as milk, cream and ice cream, are stabilized by homogenization to reduce the mean fat globule size of the dispersed phase. Water-in-oil emulsion spreads are stabilized by shearing to reduce the water droplet size and by simultaneous cooling to entrap the dispersed water droplets in the continuous phase of plastic fat, mainly in the form of small β′ crystals. This technology, called scraped-surface cooling, is used to produce margarines, reduced-fat and low-fat spreads. Butter-making involves churning (i.e., shearing to break partially the oil-in-water emulsion of cream to concentrate the fat to 80–82% by removing the aqueous phase as buttermilk). Thus, buttermilk is essentially skim milk enriched in fat globule membrane material (McCarthy and Headon, 1979). Further churning reduces the level of the aqueous phase to 16% water, containing 2% solids non-fat and up to 2% added salt. The dispersed water droplets are further sheared in the so-called working stage to reduce their diameter to less than 20 µm (Muller, 1952), to prevent microbial growth and the appearance of visible moisture. Dairy and blended spreads containing more than 72% fat can be manufactured using either scraped-surface cooling or churning equipment. Efforts by equipment manufacturers to produce low-fat butter using continuous churning equipment by incorporating the extra aqueous phase using an additional shearing unit have been commercially successful (Norgaard *et al.*, 1990). High-fat products (>95% fat), such as baking fats, are also processed using scraped-surface or drum cooling but the primary aim in their manufacture is the production of small β′ fat crystals for optimum functionality of these fats in baked goods (Joyner, 1953; Hoerr, 1960; Brooker, 1993). The principal ingredients of fat spreads are fat, emulsifier, milk protein, stabilizer, sodium chloride and water. Each of these affects the emulsion and processing characteristics of the final product. Other ingredients, such as preservatives, colors, and flavors, do not affect product processability. In dairy spreads, the fat source is milk fat to which can be added hard (Keogh *et al.*, 1988) or soft milk fat fractions (Verhagen and Warnaar, 1984). In margarine spreads, the fat source is usually either soybean oil or sunflower oil blended with a hydrogenated vegetable oil, typically in the ratio 3:1. Blended spreads contain a mixture of milk, vegetable, and other animal or marine fats.

9.1.3. Consumer Pressures for Change

A number of economic (price, inadequate promotional expenditure, low retail margins, lack of both brand advertising and innovations in packaging), medical (COMA Report, 1984; Surgeon General's Report,

1988; Department of Health (Ireland) Report, 1991) and social (more sedentary lifestyle, breakdown in traditional meal patterns, decline in the level of home cooking, wider choice of foods, decrease in bread consumption, and increased use of refrigerators, which highlighted the poor spreadability of butter) factors, have contributed to the decline in butter consumption. The development of spreads has been the response by food manufacturers to these market forces.

Consumer pressures for a cold-spreadable and more nutritionally acceptable spread were met in Sweden in 1970 by the introduction of Bregott®, an 80:20 butterfat/soya oil blend, and the low-fat spread, Latt and Lagom® (60:40 butterfat/soya oil), in 1974. It is possible to produce a product that is not subject to excessive softening or oiling-off at high ambient temperatures by using approximately 50% milk fat, 25% vegetable oil and 25% hydrogenated vegetable oil. Such a blended product, called Clover®, was first marketed in the UK in 1983. This product had a monopoly in the UK until 1985 when Meadowcup® and Golden Churn® were launched by Kerrygold and Kraft, respectively. The Dairygold® blended spread was launched on the Irish market in February 1985. The first low-fat margarine in the UK was Outline®, which was marketed by van den Berghs in the early 1970s. The first low-fat blend was Gold®, which was launched in 1979 by St. Ivel (ERC Statistics International Ltd., 1987). Initially, this product was a blend of milk fat and soya oil but soon after its introduction was changed to a full vegetable oil-based product. The first low-fat product in Ireland was Dawn Light® butter produced by Kerry Group plc. Reduced-fat butter (Kerry Group plc), reduced-fat blend (Lifestyle, by Glanbia plc), low-fat margarine (Low, by Kerry Group plc) and low-fat butter from cream (Connaught Gold®, by North Connaught Farmers Co-op) are also manufactured in Ireland.

The most important developments of recent years have been in nutraceutical spread products. An olive oil-based product (Golden Olive, by Kerry Group plc), a vegetable oil product containing 8–13.5% plant stanol esters (Benecol, by Raisio Benecol Ltd, Raisio; Duchateau *et al.*, 2002; Louter *et al.*, 2002) or 14% sterol esters (Flora Pro-Active, by Unilever plc, London) and a spread containing probiotic bacteria (Tesco own-brand by Kerry Group plc; Stanton, 2003) are being marketed. An experimental 20% fat spread containing 7.5% inulin as prebiotic has also been suggested (Rooyakkers *et al.*, 1994), but, to date, claims of prebiotics in any commercial product have not been made.

9.2.   Technical Aspects of Butter Manufacture

9.2.1.   Chemical and Physical Principles

The manufacture of butter can be divided into five steps:

1. Concentration of the fat phase of milk by mechanical separation.
2. Crystallization of the fat phase of cream by cooling.
3. Phase inversion of the oil-in-water emulsion of cream by shearing.
4. Removal of buttermilk by drainage.
5. Formation of a viscoelastic water-in-fat emulsion by working.

Each of these steps is partial and some of the steps may be combined in some processes. Only the basic principles are outlined here; for further details, the reader is referred to manuals such Anderson (1986) and Bylund (1995).

The fat content of cream is typically in the range 30–55% for batch churning systems but in a narrower range of 38–42% for continuous systems. The cream is pasteurized at 85–110°C for 10–30 s, which results in the survival of few microorganisms. Because of the high temperature treatment, the cream acquires a beneficial cooked flavor and some antioxidant properties due to the activation of free sulphydryl groups in the whey proteins. Some gas bubbles and any volatile odors present are also eliminated. Alternatively, the cream may be fermented (ripened or soured) after pasteurization using a culture of *Lactococcus lactis* subsp. *lactis, Ln. lactis* subsp. *cremoris* or a citrate-positive strain of *Lactococcus lactis* to reduce the pH to 4.5–5.0 by generating lactic acid. *Leuconostoc mesenteroides* subsp. *cremoris* or *Ln. mesenteroides* subsp. *citrovorum* simultaneously produce other desirable flavor compounds, principally diacetyl (1–3 mg/kg). In the process developed at the Netherlands Institute for Dairy Research (NIZO) (van den Berg, 1982), instead of fermenting the cream, flavor compounds and lactic acid from selected starters and starter permeate, respectively, are injected into the butter at the working stage.

The cream is cooled to 2–5°C for at least 4 h (ideally 24 h) to allow crystallization of the fat. At 5°C, the solid fat content of milk fat, determined by NMR, is in the range 50–60%.

Butter-making by churning (steps 3–5 above) involves shearing at a low temperature, which partially breaks the oil-in-water emulsion of the cream and after drainage of the buttermilk, concentrates the fat to 80–82%. After drainage of most of the buttermilk, the butter is sheared further (worked) to produce a viscoelastic water-in-fat emulsion. The objective is to reduce the aqueous phase to a maximum of 16% water, containing 2% solids non-fat with the optional addition of up to 2% salt and/or acid/flavor concentrates.

The controlling variables of the churning process are:

1. Fat content, globule size and pH of the cream
2. Cream temperature, time for crystallization and wash water temperature
3. Shear rate (agitation speed, degree of churn fill, dimensions and shape of churn)

The aim should be to control all the process variables within narrow ranges, so that the butter-making process is consistent.

A high fat content in the cream aids inversion of the emulsion, facilitating churning. The breed of cow and the stage of lactation are the main factors that affect fat globule size. Fat globules are larger and churning is easier in cream from Jersey/Guernsey milk and from milk from herds in early to mid-lactation than in late lactation. As the pH decreases, the fat globules membranes become weaker, so that fermented cream is more easily churned than sweet cream.

The churning temperature (typically 4–5°C for batch or 8–9°C for continuous churning) and the degree of fat crystallization affect most aspects of the process. A high churning temperature reduces churning time, increases fat coalescence and the ratio of free to globular fat, which in turn affects the visible moisture, texture, firmness and color of the final product. The churning time and the degree of fat coalescence affect the size of the butter grains, which should be in a certain range for optimal results. Small grains can be lost in the buttermilk, which typically contains 0.45–0.65% fat. Smaller grains also have a larger surface area, which adsorbs large amounts of aqueous phase on the surface. Conversely, large grains hold too much aqueous phase within the grains. Larger grains have a smaller surface area, making it difficult to incorporate added salt. The texture of the butter can vary from soft, crumbly or brittle to sticky or greasy due to the ratio of free to globular fat. The degree of working affects visible moisture, surface moisture, color and texture.

The shear rate during churning is governed by the speed of agitation, degree of churn fill, churn dimensions and shape. When cream containing crystallized fat is agitated during whipping or churning, fat globules form clusters, which subsequently surround the incorporated air bubbles. Some of the fat globules break due to friction and liberate their fat as free fat, which in turn destabilizes the foam, leaving buttermilk surrounding, or incorporated into, the butter grains. Higher temperatures result in more free fat, which causes the foam to break more easily. The higher the proportion of free fat, the more greasy the butter at high temperature and harder at low temperature. As churning progresses, more buttermilk is incorporated into the butter grains, thus reducing the fat lost in the buttermilk. These dispersed

water droplets are sheared further during working to reduce their maximum diameter to less than 20 μm (Muller, 1952), to prevent microbial growth and the appearance of visible moisture.

In continuous butter-making machines (Figure 9.1) that are enclosed, altering the flow rate of the cream pump and the speed of the agitation shaft (1000–2500 rpm) affects the churning time. The moisture content of butter reaches a minimum at a certain intermediate speed. At the end of churning cylinder 1, butter grains begin to appear. Optimum grain size is obtained in cylinder 2 by altering the speed of the paddles or augers from 20 to 90 rpm. After removing part of the buttermilk through outlets in cylinder 2 (or at the front of the inclined cylinder 4 in some machines), the butter grains and remaining buttermilk are sheared in the working sections by variable speed augers 4 and 6. The moisture content of the butter increases with auger speed. Secondary drainage of buttermilk can be facilitated through small perforations in the base of the worker cylinders under the augers. The butter is finally forced through a series of perforated plates in cylinder 7, when salt slurry/acid/flavor compounds may be injected after the first plate, followed by final working through the remaining plates. Typically, the moisture content after the first perforated plate is 14%, so that 2% water and 2% salt can be added as a 50% salt slurry to give butter of the desired final composition. In the NIZO process, in which acid and flavor compounds are added, the maximum moisture content at this stage is 13.5%.
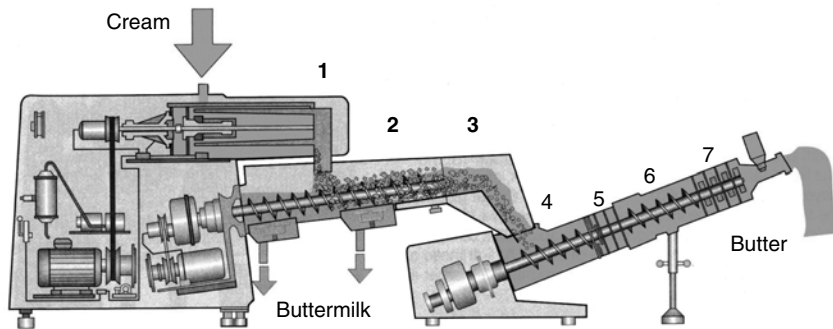

Figure 9.1.   Diagram of a continuous butter-making machine. Churning cylinder 1; separating section 2; squeeze-drying section 3; second working section 4; injection section 5; vacuum working section 6; final working stage, 7. [From Bylund, G. (1995). Butter and dairy spreads. In: *Dairy Processing Handbook,* 2nd edn, p. 274, Tetra Pak Processing Systems AB, Lund, Sweden.]

In continuous churns, the working force, F, used to shear the butter can be expressed as $F \propto \eta \cdot dv/dx$, where $\eta =$ viscosity of the butter and dv/dx is the velocity gradient. At constant viscosity, $F \propto dv/dx$ (i.e., proportional to the throughput rate and inversely to the size of holes in the perforated plates), since viscosity and the velocity gradient are inversely related. In countries with marked seasonality of milk supply (e.g., Ireland and New Zealand) the viscosity of cream is higher in winter than in summer since the fat globules are smaller and more numerous and the solid fat content is higher. In winter, cream temperature can be increased by $1°C$ to reduce cream viscosity, thereby increasing the velocity gradient and the degree of working of the butter.

After churning and during packing, the temperature of the butter should not exceed $14°C$ (Anon, 1978), because slow crystallization and further structure development during cold storage will lead to a firmer product. Aluminum foil-laminated materials are used to wrap butter, as they fully protect the fat from light-induced oxidation and associated off-flavor development.

9.3. Technical Challenges in the Processing of Fat Spreads

9.3.1. Rates of Microbial Growth

Fat spreads, whether of the high-fat or low-fat type, are usually water-in-oil plastic solids (Bullock, 1966). Microorganisms that survive the heat treatment applied prior to scraped-surface cooling are confined by the small size of the water droplets. In butter, living bacteria were not observed in droplets less than $20\,\mu m$ in diameter (Muller, 1952). Poorly-worked spreads that supported bacterial growth, had moisture droplets ranging from 50 to $100\,\mu m$ in diameter. Some growth of microorganisms is expected to occur in droplets between 20 and $30\,\mu m$ diameter, depending on the size and oxygen requirements of the organism but droplets should, in general, not exceed $30\,\mu m$ and ideally be less than $20\,\mu m$ in diameter (Bullock and Kenney, 1969). The bacteriostatic effect of 1% NaCl in a 40% fat spread containing about 53% water is much less than 2% NaCl in an 80% fat butter containing 16% water. In contrast, microorganisms are relatively free to grow and multiply in oil-in-water spreads, which have a different texture and melt-down and lack freeze-thaw stability (Tobias and Tracy, 1958; Weckel, 1965; Seas and Spurgeon, 1968).

The poor correlation between bacterial growth rate and droplet size is due partly to the limitations of the light microscope method used (Dolby, 1965). Other influencing factors are nutrient level, salt and pH of the droplet

contents. The light microscopy method involves viewing a sample of the spread under a cover glass, which distorts to some extent the droplet appearance at the spread surface. More recent methods (Juriaanse and Heertje, 1988), using electron microscopy after exposure of a fresh internal surface of the spread by freeze-fracturing, are more accurate. These latter methods have been coupled with image analysis and counting techniques, which give a true distribution of droplet size. However, because of the commercial value of these results, nothing has been published on the application of the combined techniques to fat products (Brooker, 1992 personal communication). Electron microscopy has been used successfully to study the fat crystal shell surrounding the droplets (Heertje *et al.*, 1987; Chawla *et al.*, 1990) and to determine the ion content of the droplets using X-ray spectroscopy after electron bombardment of the spread surface (Brooker, 1990).

9.3.2. Phase Inversion

It has been shown by light microscopy (Keogh *et al.*, 1988) and by scanning electron microscopy with freeze etching (Brooker, 1990) that if droplets exceed 30 μm in diameter, some will join together to form channels or 'lakes'. Eventually, the water phase may become continuous during processing (i.e., inversion of the emulsion may occur). At the other extreme, if the water droplets are too small or are over-stabilized, the phase inversion required in the mouth under the influence of shear and added aqueous phase (saliva) will not take place, or will occur too slowly. Such spreads will have an unpleasant gummy mouth-feel. The cooling sensation due to the effect of melting fat will also be absent.

9.4. Technology of Spread Manufacture

9.4.1. Processing

A scheme for the continuous processing of fat spreads is outlined in Figure 9.2. This comprises two steps, namely preparation by stirring of an aqueous phase-in-oil emulsion, followed by pumping the emulsion at a certain throughput (TP) using one or two scraped-surface coolers in series at a defined agitation rate (AR) and at a defined refrigerant temperature (RT) to form a plastic fat product with dispersed water droplets. Pin-working or crystallizing units are usually sited after each scraped-surface unit. In these units, the product is sheared, without cooling, by pins on the central rotating shaft and by static pins on the inner wall of the cylinder. The process variables, TP, AR and RT, have been studied on a pilot-scale plant (Keogh *et al.*, 1988). To produce a high-moisture spread of the water-in-oil type, the emulsion must
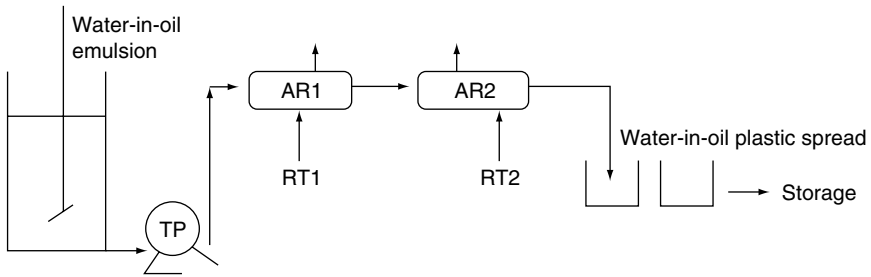
Figure 9.2. Line diagram showing variables for the production of fat spreads
TP: Pump throughput
AR1: Agitation rate, scraped surface cooler no.1
AR2: Agitation rate, scraped surface cooler no.2
RT1: Refrigerant temperature, scraped surface cooler no.1
RT2: Refrigerant temperature, scraped surface cooler no.2

be worked more intensively (850–1050 rpm) at a higher refrigerant tempera-
ture ($-2°C$ to $+4°C$) and at a lower throughput (maximum 75 kg/h) than
margarine. Under-working or over-working causes inversion of the emul-
sion. When softer fats than milk fat are used for processing, optimum results
are obtained at a lower throughput. Buchheim and Frede (1994) reviewed
other variations of the butter churning and margarine scraped-surface
cooling technologies. Frede and Buchheim (1994) reported valuable obser-
vations, based mainly on microscopy, on cultured butter, cream tempering
(physical ripening) and cream destabilization (phase inversion). It was dem-
onstrated that butter made by the churning process could be produced with
a spreadability close to that of a soft margarine, using a cream prepared
by blending low-melting and high-melting point milk fat fractions in the
ratio 60:40. Hoffmann (1989) has reviewed the role of the scraped-surface
crystallizing units and resting tubes used for margarines and shortenings. A
chapter on margarine and shortening technology by Bell (1991) concentrated
on historical developments while those by Munro *et al.* (1992) and Lane
(1992) were devoted to milk fat products.

Technical and product information is also available from the three
main suppliers of scraped-surface equipment in Europe, namely Gerstenberg
and Agger (Denmark), Schröder (Germany) and Johnson (UK) who fabri-
cate Perfector, Kombinator and Votator equipment, respectively. Numerous
patents on fat spreads have also been published, but their review is outside
the scope of this chapter.

9.5.   Fundamental Aspects of Emulsions

9.5.1.   Emulsions: Theory, Rheology and Stability to Inversion

An understanding of the mode of action of the component ingredients of fat spreads during processing requires a fundamental knowledge of the theory, rheology and stability—especially to inversion—of emulsions.

9.5.1.1.   Emulsion Theory

Liquid emulsions are inherently unstable to a varying degree. It is important to understand, therefore, the mechanisms that contribute to emulsion stability. Before the solidification step, instability of an emulsion can arise due to either phase separation or phase inversion (Mulder and Walstra, 1974). It is evident that the likelihood of phase inversion will increase as the fraction of dispersed phase increases. The vast majority of literature references are concerned with the stability to phase separation as coalescence or creaming in oil-in-water emulsions (Halling, 1981; Jaynes, 1983). In addition, a method for determining the stability of water-in-oil emulsions to inversion has not been reported. It is usually assumed that certain aspects of oil-in-water emulsion theory apply in reverse to water-in-oil emulsions.

9.5.1.2.   Electrostatic or Charge Stabilization

This theory is based on the interactions between charged solid colloidal particles in an aqueous phase. The mechanism was first described in 1941 by B.V. Derjaguin and L. Landau and in 1948 by E.J.W. Verwey and J.Th.G. Overbeek and is referred to as the DLVO mechanism after them (see Bergenståhl and Claesson, 1990). Charged particles in suspension attract oppositely charged ions from the immediate environment to their charged surfaces to form a layer of counter-ions. This, in turn, leads to the formation of a further secondary layer of opposite charge to the first layer—the so-called electrical double layer. Thus, two charged particles give rise to a repulsion potential, which depends on the distance between them and the height of the potential. If the repulsion potential is greater than the attraction potential, the particles will be stable. The attraction potential the van der Waals' interaction, has as its source, the rapidly fluctuating dipole moment ($\sim$105/s) of a neutral atom, which induces a polarized charge in neighbouring atoms, resulting in an attraction. The extent of the attraction is proportional to the polarizability of the atoms, divided by the distance between them. A feature of the theory is the rapid increase in attraction potential at small distances. The sum of the repulsive and attractive potentials

gives the total potential, which determines the stability of the system. For very short distances, the attraction potential is always the greater and aggregation results. For intermediate distances, the repulsive potential, if large, results in a stable system. The most useful aspect of the DLVO theory is its ability to explain the destabilizing effect of added neutral salts, which compress the double layer and reduce the repulsive potential, resulting in flocculation. According to the Schulze-Hardy rule, the ratio of critical flocculation concentration for monovalent, divalent and trivalent ions is 100:1.6:0.13. Thus, divalent ions are over 50 times more effective than monovalent ions in destabilizing colloids (Friberg *et al.*, 1990).

It is important to state that while the DLVO theory is a useful starting point, it applies to solid, charged particles in an aqueous suspension but does not apply accurately to emulsions, which are liquid systems. Also, the mechanism does not apply to water-in-oil emulsions, in which the repulsive layer is located on the concave side of the interface and, therefore, is too distant from a neighboring droplet to exert a repulsive force. Moreover, since the continuous phase of a water-in-oil emulsion is non-ionizing, the development of an electrical double layer is not to be expected (Sherman, 1955b). In addition, the monoglyceride emulsifiers normally used in the manufacture of spreads are non-ionic. Conversely, the charge on protein molecules is quantifiable by the zeta potential and depends on the pH of the suspending medium, as well as on other ions. However, proteins such as sodium caseinate bind cations, making the prediction of the emulsion behavior very difficult in anything more than a qualitative way. Thus, oil-in-water emulsions stabilized by $\alpha_s(\alpha_{s1} + \alpha_{s2})$-casein follow the DLVO theory and are reversibly flocculated by increasing ionic strength but similar emulsions stabilized by β-casein behave differently (Dickinson *et al.*, 1987).

9.5.1.3. Steric Stabilization

Flexible macromolecules, such as proteins, and small molecules, such as surfactants, are amphipathic and may form a layer at the oil-water interface. These molecules may also partly stabilize emulsions by forming a physical barrier to close contact, thereby reducing the attractive van der Waals' forces to ineffective levels (Dalgleish, 1989). Repulsion can arise in either of two ways and physico-chemical calculations are available for both mechanisms in oil-in-water systems. Either the approaching protein-coated particles will tend to compress or alternatively interpenetrate the adsorbed protein layer on adjacent particles. The optimum structure of the stabilizing protein will be dealt with in the section on protein as an ingredient.

9.5.1.4.   Solid Particle Stabilization

In a few instances, emulsions can be stabilized by solid particles as shown by S. U. Pickering in 1907 (see Petrowski, 1976). Examples are mustard seeds in traditional mayonnaise (Anon., 1968) or crystals of high melting-point triglycerides in margarine or butter (Precht and Buchheim, 1980; Heertje *et al.*, 1987). The solid particles are considered to act as a barrier, which prevents the coalescence of droplets. Bancroft (1913) observed that the phase that wets the solid more easily will become the continuous phase.

Traditional mayonnaise is an 80% oil-in-water emulsion, which may rely on mustard seeds for solid particle stabilization. Two constituents of egg yolk, lecithin and cholesterol, are surfactants, which promote the formation of oil-in-water and water-in-oil emulsions, respectively. The ratio of lecithin to cholesterol in egg yolk favors the water-in-oil type but the final emulsion type formed is due to the action of mustard seed, which favors an oil-in-water emulsion (Petrowski, 1976).

9.5.1.5.   Stabilization by Rheological Methods

Increasing the viscosity of the continuous phase in an oil-in-water emulsion increases stability to flocculation, coalescence and oiling-off by reducing the frequency of collision between droplets. However, the kinetic theory of flocculation suggests (Friberg *et al.*, 1990) that the effect of the viscosity of the continuous phase is less than expected without the concurrent energy barrier provided by electrostatic and/or steric effects. In non-food water-in-oil systems, the viscosity of the continuous phase can be adjusted by choice of hydrocarbon oil of varying molecular weight or by addition of oil-soluble compounds of high viscosity. However, in water-in-oil emulsions, there are no indications of the effects of the viscosity of the internal aqueous phase on the stability of emulsions to oiling-off or inversion (Sherman, 1950). The higher the viscosity of the aqueous phase in multi-ingredient food systems containing protein and starch, the higher the stability (Platt, 1988). In this case, stability may be due to effects associated with the viscosity generated by the ingredients (which will be referred to again below) rather than to the effect of viscosity *per se*. On the contrary, Sherman (1955a) showed that the viscosity of the aqueous phase had no effect on the viscosity of water-in-oil emulsions and, therefore, should not affect emulsion stability.

In earlier work (Sherman, 1950), inversion of water-in-oil emulsions was achieved by increasing the proportion of the dispersed aqueous phase. Emulsions containing 2–5% non-ionic emulsifier and up to 50% water are Newtonian. Above this level of water, the rheological parameters increased significantly and inversion occurred at a water concentration of 75–80%. The

higher the concentration of emulsifier, the higher the viscosity before inversion. A large decrease in viscosity accompanied inversion to an oil-in-water emulsion. The viscosity of the inverted oil-in-water emulsion should be of the same order but slightly less than that of a water-in-oil emulsion because the continuous phase dominates the viscosity at identical ratios of dispersed phase. Thus, since water has a viscosity of 1 mPa s, while that of hydrocarbon oils is of the order of 25 mPa s, the oil-in-water emulsion at the same level of dispersed phase will have a slightly lower viscosity. Since there are few other relevant references devoted to water-in-oil emulsion theory (Sherman, 1955c; 1967a; 1967b), one must return to the view (quoted in many reviews on emulsions) that oil-in-water emulsion theory applies in reverse to water-in-oil emulsions. References outside the patent literature on fat spreads are not available on multicomponent water-in-oil emulsions using food ingredients.

9.5.1.6. Rheology of Water-in-oil Emulsions

According to Sherman (1955a,b), the factors that affect the rheological characteristics of a water-in-mineral oil emulsion are:

- *Volume of the dispersed phase*. Below a concentration of 50% aqueous phase and with 2–5% non-ionic emulsifier, emulsions behave as Newtonian fluids; above 50% aqueous phase, emulsions become increasingly non-Newtonian (i.e., become shear rate-dependent and develop a yield value).
- *Viscosity of the dispersed phase*. The viscosity of the dispersed phase that ranged from 11 to 560 mPa s, did not affect the viscosity or yield value of the emulsion. The disperse phase fraction, φ, was 0.717.
- *Viscosity of the continuous phase*. The viscosity of the continuous or oil phase is related directly to emulsion viscosity and yield value. In non-food systems, oil viscosity can be varied by choosing hydrocarbon oils of varying molecular weight or by adding of viscous, oil-soluble compounds. In addition, as already mentioned, emulsion stability is increased by reducing the rate of globule flocculation in a continuous phase of higher viscosity.
- *Type and concentration of emulsifier*. The viscosity and yield value of emulsions ($\varphi_{vol} = 0.66$) depend on the chemical nature of the emulsifier. Sherman (1955c) proposed two possible reasons for this, namely interfacial viscosity and interfacial adsorption. Interfacial viscosity affects the resistance of droplets to deformation, which is reflected in the resulting emulsion viscosity. A high level of interfacial adsorption enlarges the size of the interfacial layer significantly and increases emulsion viscosity. Adsorption of emulsifier at the interface should also increase with the concentration of emulsifier. The

increase in the overall dimensions of the resulting droplets increases the viscosity and yield value.

- *pH*. In a study in which a fatty acid ester emulsifier was used, water-in-oil emulsions were stable up to pH 9.0 (Sherman, 1955c). Above this value, inversion occurred either through interaction between ester and alkali or through soap formation between alkali and contaminating free fatty acids, which causes solubilization of the ester emulsifier.
- *Ionic conditions*. The influence of certain salts and metallic oxides used in printing fluids that affect the rheological properties of the aqueous phase and emulsions were studied by Sherman (1955b). As the conditions are not relevant to foods, ionic conditions will be dealt with later under ingredient effects.

9.5.1.7.   Stability of Water-in-oil Emulsions to Inversion

The effects of the concentration of the dispersed phase, emulsifier type and concentration on the inversion of water-in-oil emulsions have been described above. However, since in this chapter, it is proposed to examine the effects of a multi-ingredient system on emulsion stability, each of the following factors must be considered: emulsion formation, stability, instability, effects of shear and effects of ingredients (Becher, 1977).

9.5.1.8.   Emulsion Formation

The method of emulsion formation must be standardized and reproducible. The equipment used, the shear rate, the time and temperature of emulsification are the main factors in emulsion formation.

9.5.1.9.   Emulsion Stability

Many methods are used to measure the stability of oil-in-water emulsions. Basically, coalescence is measured by the change in emulsion droplet size with time (Halling, 1981). However, since coalescence is slow in protein-stabilized emulsions (Tornberg and Ediriweera, 1987), an accelerated method is usually used. The effects on coalescence of accelerated methods such as heating, freezing and shear stress have been measured by changes in turbidity (Pearce and Kinsella, 1978; Tornberg and Ediriweera, 1987; Melsen and Walstra, 1989). Other investigators have used measurements of particle size determined by Coulter counter (Hassander *et al*., 1989), centrifugal photo-sedimentation (Boyd *et al*., 1972), or an electronic imaging system (Klemaszewski *et al*., 1989) or by other indirect methods including microwave irradiation (Petrowski, 1974) and solvent extraction of free fat (Foley *et al*.,

1971; Tornberg and Ediriweera, 1987). Phase inversion temperature (Shinoda and Saito, 1969) and emulsifying capacity (Swift *et al.*, 1961) have been used to evaluate the effects of low molecular weight and protein emulsifiers, respectively. Unfortunately, it is not possible to measure the size of the large droplets present in unhomogenized water-in-oil emulsions because the droplets coalesce very quickly. The phase inversion temperature is not a relevant test, as it may not be related directly to the stability to inversion at the emulsification temperature. Furthermore, it has been stated (Matsumoto and Sherman, 1970) that water-in-oil emulsions do not exhibit a true phase inversion temperature, unlike oil-in-water emulsions.

9.5.1.10. Emulsion Instability

A quantitative method for assessing stability to inversion and the extent of emulsion inversion was developed by Keogh (1993). The rheological method used was based on the observation that, provided an aqueous phase with a viscosity greater than 300 mPa s at 367/s was used to prepare a 25% fat emulsion, a stable water-in-oil emulsion is slightly thixotropic, a mixed emulsion is slightly rheopectic while an incompletely inverted water-in-oil emulsion is highly rheopectic. Thus, the change in viscosity over time (240 s) at a constant shear rate (291/s) at 40°C varied from −49 mPa s for a stable emulsion to +449 mPa s for an unstable emulsion. The stability of the emulsions to inversion was also assessed visually after dispersion in cold water. The change in viscosity of a stable water-in-oil emulsion over time was negative or low (<100 mPa s), that for a mixed emulsion was intermediate (100–200 mPa s), and that for an incompletely inverted emulsion was high (>200 mPa s), as shown in Figure 9.3 (Keogh, 1993).

Measurement of the electrical conductivity of emulsions has been considered as an alternative method since oil-in-water emulsions exhibit higher conductivity than water-in-oil emulsions (Ršhl, 1972). However, this method which has been used with some success to control the level of water in butter (Prentice, 1953), has the disadvantage of being dependent on ion concentration. Therefore, certain added ions increase conductivity but might not increase stability to inversion.

9.5.1.11. Effects of Shear on Emulsion Stability

There is very little published information on the effect of shear or stirring conditions on the stability of water-in-oil emulsions to inversion. It has been established (Keogh *et al.*, 1988) that water-in-oil emulsions (60g of a 7% sodium caseinate solution dispersed in 40 g milk fat) are stable to inversion only within a narrow range of throughput, refrigerant temperature and agitation rate when processed in a single-unit Votator scraped-surface
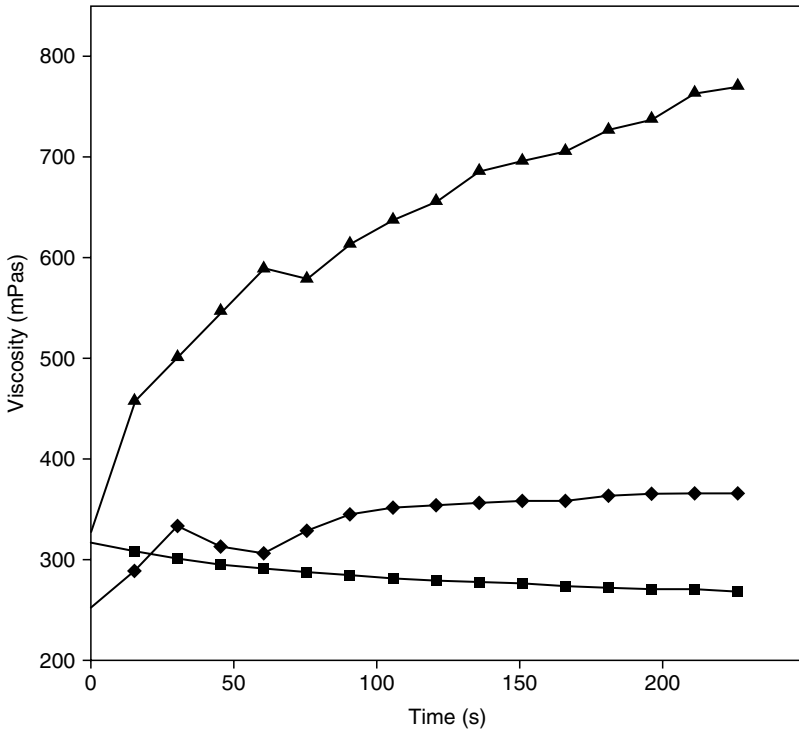
Figure 9.3.   Viscosity of an water-in-oil (■), mixed (◆) and inverted (▲) emulsions as a function time at constant shear rate.

cooler. Until recently, such information was regarded as commercially valuable and therefore was not published. Patents usually give wide ranges of processing conditions. Frequently, the conditions given, though suitable for one scraped-surface cooling system, are misleading for another system. Although guidelines can be given, processing conditions need to be optimized for each product and processing system.

9.6.   Effects of Ingredients on Emulsion Stability

9.6.1.   Fat

As already outlined, the higher the viscosity of the continuous oil phase, the higher the viscosity of the emulsion. At high emulsion viscosity, the stability to coalescence is increased, and may result in increased stability

to inversion. Fats of animal, vegetable, or marine origin contain about 99% triglycerides and non-triglyceride materials can, for the most part, be ignored. The presence of small amounts of surfactants such as lecithins, monoglycerides, and cholesterol should, however, be kept in mind. The functional behavior of a fat or blend of fats will depend on:

- The melting point (determined as slip point or drop point) of the fat.
- The content of solid or crystalline triglycerides present at any temperature but especially in the range of 4–37°C.
- The crystal form which is determined by the degree of heterogeneity of the triglycerides (i.e., of the fatty acids present in the molecule). The greater the diversity of fatty acids present, the more likely the fat is to form the less stable, smaller rectangular ($1 \times 6\,\mu m$) β' crystals rather than the larger (20–30 μm diameter), more stable β crystals, which result in a sandy mouth-feel (Mostafa *et al.*, 1985). Sunflower oil, lard, cocoa butter and coconut or palm kernel oils tend to form β crystals during storage after cooling. Milk fat is naturally very heterogeneous. Palm oil, tallow and fish oils are also heterogeneous, as is lard after interesterification and partial hydrogenation. While heterogeneity can be increased by interesterification, it is usually sufficient to increase the diversity of fatty acids by adding another fat or surfactant to prevent the formation of β crystals. It has been shown that the presence of hexadecanoic ($C_{16:0}$) (Wiedermann, 1978) or docosenoic ($C_{22:1}$) acid (Hojerobá *et al.*, 1992) is particularly effective in this regard. Therefore, crystal size and crystal form are interrelated (Hoerr, 1960).

The melting point of fats used for spreads should not exceed mouth temperature (37°C) as an unpleasant waxy effect, termed palate-cling, starts to develop above this temperature.

The proportion of solid fat at any given temperature largely determines the behavior of the fat at that temperature. The content of solid fat can be measured by dilatometry, by wide-line nuclear magnetic resonance (NMR) or by pulsed NMR. The latter method is now commonly used commercially to measure the solid fat content (SFC). The behavior of fats and fat blends during processing and storage of spreads can be related to the SFC versus temperature curve. The SFC/temperature profile of a fat is related to many characteristics of the product, including general appearance, packability (in foil wrap or tub), organoleptic properties (flavor release, "coolness" and thickness), firmness/spreadability and oil exudation. The firmness or yield value of a plastic material can be measured directly using a controlled stress rheometer or indirectly using a cone penetrometer (Haighton, 1959), extruder (Prentice, 1954) or sectilometer (Knoop, 1972). Haighton (1959) developed a formula

to relate the penetration depth to the yield value, but the latter two indirect methods measure the force required to extrude and cut the sample, respectively. It is important to note that the yield value is related to, but is not equivalent to, spreadability. The reason is that when the yield value is being measured in shear flow, the velocity gradient or rate of shear is perpendicular to the direction of the stress applied. In elongational flow, the rate of shear is in the direction of the stress applied. When spreading by hand, the rate of shear lies somewhere between elongational and perpendicular flow (van Vliet, 1991). The SFC at 4 or 10°C is related to the spreadability ex-refrigerator; an SFC of not greater than 32% at 10°C should result in a butter that is spreadable at 4°C (i.e., having a maximum yield value of $1000 \, g/cm^2$ at 4°C). Packability can be assessed by the yield value at 15°C. A minimum yield value in the region of $500 \, g/cm^2$ is desirable for foil wrapping. The SFC at 20°C determines the tendency towards oil exudation; a value of at least 10% is essential to prevent oiling off. This value represents the level of fat crystals required to adsorb the free oil present in the product at this temperature.

Butter that is "fridge-spreadable," but with poor stand-up qualities at 21°C, was made from combinations of mainly the low melting liquid butter-oil fraction and some of the high melting solid fraction. In addition, the use of a very high melting-point solid fraction, obtained by acetone fractionation, enabled the production of butter, which was also physically stable at 21°C. In addition to their illegal status, the fractions crystallized from solutions in acetone were also more susceptible to oxidation (Kaylegian and Lindsay, 1992). A more acceptable means of obtaining very high melting fractions is therefore required.

In low-fat spreads, margarines and shortenings, a proportion of high melting-point fats is necessary for the formation of the fat crystal network (Haighton, 1976). Aggregation of the crystals occurs to form platelets and eventually shells that surround the water droplets (Precht and Buchheim, 1980; Heertje *et al*., 1987; 1988; Juriaanse and Heertje, 1988; Chawla *et al*., 1990). As already mentioned, the crystals should preferably be in the small β′ form. The absence of β′ crystals may lead to a lack of plasticity and the possibility to inversion during the cooling process but has no direct effect on the emulsion at 40°C. However, during the preparation of emulsions, care must always be taken to melt completely crystals of fats and emulsifiers. The presence of large crystals could rupture the droplets during both stirring and cooling of the emulsion and thereby cause inversion.

9.6.2. Emulsifiers

Emulsifiers reduce the interfacial tension between two mutually-immiscible liquids because they have an affinity for both phases. Ford and

Furmidge (1966) indicated three essential properties of an emulsifier for concentrated water-in-oil emulsions:

- The emulsifier should reduce the interfacial tension to less than 1 mN/m.
- It should form a relatively rigid, uncharged interfacial film, through either electrostatic or hydrogen bonding between the emulsifier molecules, which will prevent the coalescence of water droplets while facilitating the coalescence of oil droplets.
- It should adsorb rapidly at the oil/water interface.

Sherman (1973) suggested that a strong interfacial film of emulsifier is also necessary to prevent the coalescence of water droplets in oil-continuous emulsions.

The emulsifier should always be more soluble in the continuous than in the dispersed phase (Bancroft, 1913). Solubility is related to polarity, which has been rated on a hydrophilic to hydrophobic scale of 20 to 1 by (Griffin, 1949, 1954). Ford and Furmidge (1966) noted that water-in-oil emulsifiers have a hydrophilic:lipophilic ratio (the so-called HLB) of 3.5 to 6.0 but interfacial viscoelasticity was not quantified. The emulsifiers used were fatty acid monoesters of either sorbitol or glycerol. Later, it was shown empirically that monoglycerides with a HLB value in the region of 3–4 and a degree of unsaturation corresponding to an iodine number of 40–55 give good stability in water-in-oil emulsions (de Feijter and Benjamins, 1978). The properties of a range of monoglyceride emulsifiers of different iodine number, fatty acid profile, HLB and slip point are summarized in Table 9.1. Monoglycerides with an iodine number of 40–55 and a melting point in the region of 55°C were found to be optimal (Grindsted, 1988) because this level of unsaturation conferred an ideal level of flexibility/rigidity on the molecule (Garti and Remon, 1984). Variations in fatty acid composition have only a slight effect on HLB but quite a marked effect on emulsion stability (Quest International, 1988). In water-in-oil emulsions, there is a positive correlation between emulsion stability and fatty acid chain length and a negative correlation with the dielectric constant of the emulsifier (Goubran and Garti, 1988). Increasing molecular weight and decreasing dielectric constant indicate greater hydrophobicity, which leads to greater impregnation of the interface and to a more stable emulsion.

9.6.3. Proteins

Proteins can affect the stability of emulsions by electrostatic, steric or rheological means, as already described. The mechanisms involved are highly complex, interactive and, as a result, are very difficult to quantify.

Table 9.1. Properties of partially hydrogenated monoglyceride emulsifiers

Commercial name[a]	Iodine number	Saturated fatty acids (%, w/w)	Unsaturated fatty acids (%, w/w)	HLB	Slip Point (°C)	Source
Dimodan S	40–50	50.4	47.7	4.3	61	Lard
Dimodan OT	55	22.2	76.8	4.3	55	Tallow olein
Dimodan CP	80	28.9	70.3	4.3	48	Vegetable oils
Dimodan LS	105	–	–	4.3	44	Sunflower oil

HLB = hydrophilic-lipophilic balance.
[a] Grinsted Products A/S, Brabrand, data sheets.

However, valuable insights into their mode of action have been obtained (Dalgleish, 1989; Leman and Kinsella, 1989).

In order to adsorb at the interface and be surface-active, proteins must be flexible and amphipathic (i.e., have some affinity for both phases). The hydrophobic side-chains will adsorb on the oil side of the interface while the hydrophilic side-chains will interact with the aqueous phase. Three aspects of the side-chains of a protein are important:

- Content, location and grouping of the hydrophilic and hydrophobic amino acid residues.
- Surface polarity or charge that is relatively more important for emulsions than the overall charge.
- Protein structure and ability to unfold that are determined by the amino acid sequence.

Proteins are dynamic molecules with respect to structure. The preferred "folded" structure for a given set of environmental conditions is that which has the minimum free energy. The driving force to assume a given folded structure is a thermodynamic force. In aqueous systems, the hydrophobic side-chains will endeavour to orient away from the surrounding water and towards the core of the molecule. However, for high surface activity, it is essential that the protein molecule should unfold and orient its hydrophobic side-chains towards the oil phase. A lack of hydrophilic residues usually does not restrict protein functionality at interfaces. Thus, flexible proteins can create a highly hydrated, mobile layer to stabilize an emulsion particle.

One way of increasing the flexibility of a protein is to denature it (e.g., by heating or treatment with urea). Denaturation will also occur after adsorption of the protein at the interface (Graham and Phillips, 1979), a process that may be slow. The properties of an emulsion are, therefore, time-dependent.

The third mechanism by which proteins affect the stability of emulsions is rheological. This mechanism derives fundamentally from electrostatic and steric effects. The importance of viscosity has been described earlier. The viscosity of a caseinate solution is, *inter alia*, an indicator of the degree of bound water absorbed by the hydrophilic groups, as well as the water trapped inside the molecular aggregates (Korolczuk, 1982). The viscosity parameters (K, apparent viscosity at zero shear stress; n, the power law factor and σ_y, the yield stress) of sodium caseinate have been studied and found to be affected by concentration (Hermansson, 1975), precipitation and solution pH of caseinate (Hayes and Muller, 1961; Korolczuk, 1982), denaturation (Hayes and Muller, 1961; Canton and Mulvihill, 1982), sodium chloride (Hermansson, 1975; Creamer, 1985), calcium chloride (Hayes and Muller, 1961) and temperature (Korolczuk, 1982).

9.6.4. Hydrocolloid Stabilizers

From a rheological and stability standpoint, hydrocolloid stabilizers may be used to increase the viscosity of the aqueous phase of a low-fat spread by binding water. Gelatin is of special interest, as it is a protein. However, the lack of hydrophobicity of gelatin gives it a low rating in terms of interfacial activity and consequent emulsion stability (Chesworth *et al*., 1985). It has been shown that caseinate displaces gelatin at the oil/water interface (Dickinson *et al*., 1985). At high ratios of caseinate:gelatin, gelatin is desorbed entirely and is found only in the aqueous phase (Musselwhite, 1966). Thus, gelatin has no electrostatic or steric role at the interface in a water-in-oil emulsion but has a role in increasing viscosity through water binding.

Other hydrocolloids that increase the viscosity of aqueous systems are also potentially useful. These include carrageenans and starch. κ-Carrageenan is an anionic polysaccharide, which reacts with positive sites on κ-caseinate (Snoeren *et al*., 1976). This interaction can occur even when the pH of the solution is above the isoelectric point of the protein (Bettelheim *et al*., 1966) and has been attributed to the uneven distribution of charged residues on the protein. The interaction seems to be electrostatic because it is eliminated by chemical modification of the caseinate to remove all positive sites (Day *et al*., 1970). The maximum effect of κ-carrageenan occurs at lower ratios. The ratio of κ-carrageenan to caseinate should not exceed 1:4 as some precipitation of the aggregates occurs (Elfak *et al*., 1979). Interaction between other carrageenans (λ and ι) and α_s-(α_{s1}-, α_{s2}-) and β-caseins *via* Ca^{2+} bridging also seems to occur, since these proteins bind carrageenans in the presence of Ca^{2+} (Hansen, 1968), and the casein-carrageenan interaction is minimal in the absence of Ca^{2+} (Lin and Hansen, 1970).

Starch is also of interest because it is reported to increase the viscosity of caseinate solutions in a synergistic manner, especially at caseinate concentrations > 10% (Platt, 1988). The increase in viscosity was thought not to be due to any chemical interaction (Jones and Wilson, 1976) but rather to the increase in the swelling volume of the starch (Lelièvre and Husbands, 1989).

9.6.5. Sodium Chloride

The effect of Na^+ on the stability of water-in-oil emulsions is exercised mainly through its influence on sodium caseinate. It has been shown that as the surface concentration of casein on oil droplets is increased, the oil-in-water emulsion becomes less susceptible to flocculation/coalescence in the presence of electrolyte. Added NaCl broadens the droplet size distribution at a low casein content (0.25%) but causes this effect at a high casein content (0.5%) only when $CaCl_2$ is added (Dickinson et al., 1984).

9.6.6. Disodium Phosphate and Trisodium Citrate

These salts have little effect on the water-binding capacity and viscosity of caseinate. Their main effect is to bind any residual calcium in the system (Vakaleris and Sabharwal, 1972).

9.6.7. pH

The effects of pH on caseinate have been discussed above.

9.6.8. Interactions of Ingredients

The interactive behaviour of proteins, such as sodium caseinate, and small molecule surfactants, such as monoglycerides, is very important for emulsion stability. The competitive displacement behavior of some food proteins, including casein and its fractions, has been described above. Caseinates and monoglycerides adsorb competitively at the oil-water interface. The main difference is that monoglycerides, being much smaller (mol wt., ~ 380 Da) adsorb more quickly at the interface. Information has been generated, mainly through studies on interfacial tension and more recently using confocal laser scanning microscopy.

The ability of starch, specifically amylose, to complex with l-monoglycerides may modify the activity of the monoglyceride in some way. On a weight basis, the maximum complexing ability was found with the monoglyceride of tetradecanoic acid while on a molar basis, maximum complexation occurred with the monoglyceride of octadecanoic acid. For monoglycerides derived from unhydrogenated soya oil (55% $C_{18:3}$) and

hydrogenated soya oil ($85\%C_{18:0}$) the complexing indices were 28 and 87%, respectively (Krog, 1971). Takeo and Kuge (1969) showed that the inner diameter of an amylose helix can vary from 4.5 to 6.0Å. The inner surface of the helix is lipophilic in character, but the diameter determines the size of monoglyceride molecule, which can enter. It was shown by Krog (1971) that molecules with a large polar group are poor amylose-complexing agents while molecules that have a straight hydrocarbon chain and a small polar group, such as saturated monoglycerides, are very effective complexing agents.

9.7. Interactions of Ingredients in Low-Fat Spreads

In low-fat stirred emulsions, the composition and rheological properties of the aqueous phase were shown to affect the stability of the emulsion to inversion (Keogh, 1993). The principal effects and interactions of the various ingredients were complex, but of very practical and commercial significance. The levels of each ingredient chosen for study are indicated in Table 9.2. When the effect of constant shear rate over time on emulsion viscosity is measured, the effect due to droplet size should also be considered. It was shown (Sherman, 1983) that viscosity increases as mean droplet size decreases but the effect is small for droplets of mean diameter $>3.3\,\mu m$. The effect of droplet size, therefore, is significant in homogenized emulsions but can be ignored in stirred emulsions in which the mean droplet size is much larger.

The effect of sodium caseinate, alone (Hermansson, 1975), and with starch (Platt, 1988) or NaCl (Hayes and Muller, 1961; Hermansson, 1975), on the viscosity of an aqueous system is well documented in the literature but

Table 9.2. Ingredients and levels studied in water-in-oil emulsions (Keogh, 1993)

Ingredient	Ingredient (%)	
Emulsifier[a]	0.5	0.7
Na caseinate[b]	12.0	13.0
Starch	1.33	1.67
NaCl	0.67	1.33
$Na_2HPO_4 + Na_3$ citrate	0.33 + 0.37	0.66 + 0.74
pH	5.38 – 5.69	6.60 – 6.85

[a] Percentage in emulsion.
[b] Percentage in aqueous phase.

Table 9.3. Main effects of the ingredients on the viscosity parameters of the aqueous phase of emulsions (Keogh, 1993)[a]

	Aqueous phase viscosity parameters and percentage variance							
	K(mPas)			s.s %/	n			s.s %/Total s.s[b]
Ingredient	Low	High	Significance	Total s.s[b]	Low	High	Significance	
Emulsifier	–	–	NA	–	–	–	NA	–
Na caseinate	1370	3180	***	27.7	0.85	0.80	***	13.0
Starch	1780	2770	***	8.3	0.84	0.81	***	5.3
NaCl	2020	2530	***	2.3	0.83	0.82	NS	–
Na$_2$HPO$_4$ + Na$_3$ citrate	2440	2110	NS	–	0.81	0.83	NS	–
pH	3280	1270	***	34.2	0.76	0.88	***	63.3
	LSD$_{t0.001}$ = 434				LSD$_{t0.001}$ = 0.023			

[a] K is the apparent viscosity at zero shear stress and n is the power law factor

[b] Sum of squares (ss)/total sum of squares expressed as a percentage. NA = not available;

NS = not significant; LSD$_{t0.001}$ = least significant difference (standard error of difference × Student's test value) at the 0.001 level; *** = significant at the 0.001 level

the effect of phosphate + citrate (Vakaleris and Sabharwal, 1972) and pH is less clear (Hayes and Muller, 1961; Korolczuk, 1982; Creamer, 1985). The significance of these ingredients for the stability of water-in-oil emulsions to inversion has not been published.

The addition of NaCl to the aqueous phase is mainly for organoleptic reasons but its effect on caseinate must not be overlooked. Increasing the concentration of NaCl increases the ionic strength without the complications of the strong binding phenomena found with divalent cations. Large changes in the concentration of the univalent cations and their associated anion have a number of important effects on the viscosity of the aqueous phase (Table 9.3) and the stability of the emulsion. The observed small decrease in pH and the increased viscosity of the aqueous system may be related directly.

The probability exists that swelling or partial unfolding of caseinate aggregates occurs at high NaCl concentrations, thus increasing the voluminosity of the protein and hence the viscosity of the system. The associated decrease in pH at high NaCl concentrations results from the groups exposed during swelling. The steric effects of these exposed side-chains may also increase pseudoplasticity, as well as viscosity. Increasing the concentration of NaCl from 1.33 to 2%, increased pseudoplasticity. Some binding of the residual calcium by the phosphate and citrate was shown to prevent the increase in the viscosity of the aqueous phase.

Lowering the pH to 5.4 reduces the negative charge on the protein, which results in a large increase in viscosity due to partial aggregation. According to Creamer (1985), further acidification and dissociation of calcium from casein micelles results in precipitation, dehydration and reduced viscosity below pH 5.3. Similar behavior was observed (Keogh, 1993) in low-fat emulsions stablized by sodium caseinate, although colloidal calcium phosphate is not is responsible for this phenomenon, since it has already been largely solubilized by acidification and eliminated during the manufacture of casein.

Emulsions are complex systems containing many ingredients, which interact affecting the stability and rheology of the emulsion system. Many of these interactions and effects have been studied in detail by Keogh (1993).

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