Chapter 8
Emulsifiers in Infant Nutritional Products

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8.1 Introduction

Infant nutritional products 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 will include some background information on the various types of nutritional products and before describing the role of emulsifiers in infant nutritional products, some background on the various production processes involved will be outlined, with emphasis on emulsion formation and stabilisation. The typical protein sources and low molecular weight emulsifiers available for use in these products will be 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 stabilisation of emulsions will be discussed.

8.2 Types of Infant Nutritional Products

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 are, in essence, 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

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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 60:40. The enrichment of infant formula with selected whey proteins, such as α-lactalbumin (a-lac), that are more abundant in human milk than in bovine milk has been a recent innovation.

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 pre-disposition 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, for example those 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 that develop a high viscosity in the infants stomach. These formulae contain permitted hydrocolloids such as starch or locust bean gum. 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 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 the

Table 8.1 Typical composition (per 100 ml) of some infant nutritional products

Formula type	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
First age (whey dominant)	66–68	1.4–1.7	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
Second age 'Follow on'	65–70	2.1–2.3	3.0–3.6	6.6–8.0
'Nutrient dense'	91	2.0	4.9	9.8
LBW	80	1.9–2.4	4.2–4.4	7.9–8.6
Growing up milk	100	3.5–3.7	3.3–3.5	13.6–13.8

infant with a superior nutritional source than bovine milk. Specially formulated milks are now being designed for toddlers and young children that range in age from approximately 3–7 years. These milks, commonly referred to as 'growing up milks', are similar in composition to follow-on formulae. Vanilla, chocolate and a range of fruit flavourings are commonly added to enhance the taste and aroma of these products.

In recent years, nutritional preparations for pregnant and lactating women have become available. There is a wide variety of these products 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 Stabilisation

Processes used in the manufacture of infant nutritional products 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 powders products or, sterilisation in the case of ready-to-feed or concentrated liquid products. Powdered nutritional products may be produced using three general types of processes. The first process involves dry blending dehydrated ingredients to constitute a uniform formula. The second 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. Liquid nutritional products 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. The production of recombined milk 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 formula 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) (Fig. 8.1).

Homogenisation is normally achieved in dairy processes, including the production of infant nutritional products, by conventional valve homogenisers in which fat globules are forced through a small orifice under high pressure. The combination of shear forces and impact forces reduces large fat globules into smaller one. Microfluidisation is an alternative homogenising process. In this process, the mixture enters an interaction chamber, which has fixed-geometry micro-channels that divides the product into

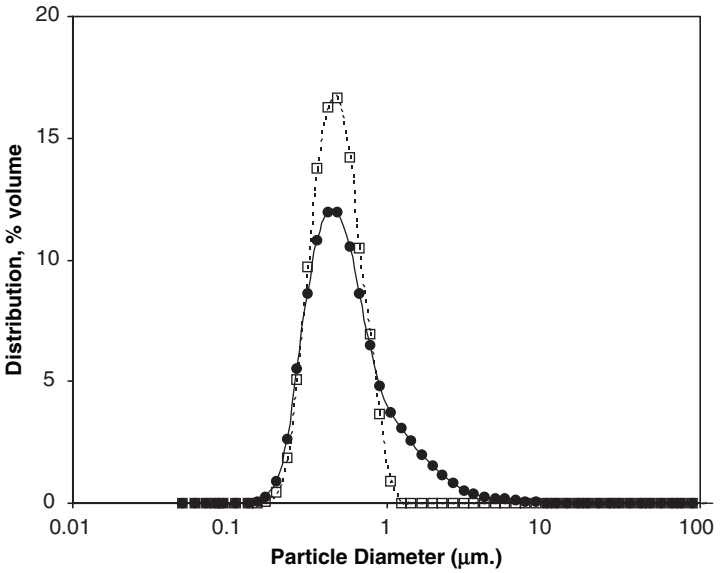

Fig. 8.1 Fat globule size distribution profile of an infant nutritional product analysed post-homogenisation, but prior to UHT-processing (■) and after UHT-processing (●)

tiny streams. The streams accelerate to a very high velocity as they flow through the interaction chamber. The system is designed such that these 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 microfluidiser is usually performed at higher pressures than is used in conventional valve homogenisation (Olson et al., 2004).

After the formation of the o/w emulsion, it undergoes sterilisation or dehydration to inactivate microorganisms. Thus, the emulsion formed must be capable of withstanding the associated pumping, shearing and thermal treatments.

In the production of powdered infant nutritional products, the o/w emulsion is normally heat treated to destroy pathogenic bacteria and evaporated prior to the dehydration step. 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). An ultra-high-temperature (UHT) treatment (e.g. 135–150 °C for 3–5 s) may be applied prior to drying. The final step involves the dehydration of the emulsion until a low moisture content, typically <3%, is achieved. Spray drying is a common large scale drying system used to dry heat sensitive powders such as infant formula. The emulsion is atomised 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 pouches. Apart from the nutritional and microbial quality, the dehydrated emulsion must be easy to reconstitute in luke-warm water and when reconstituted must be free of lumps and other defects such as free fat, greasiness and white flecks that float on the surface and adhere to the sides of the containers. 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 skim milk powder (SMP) alone, whey protein isolate (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. The reader is referred Pisecky (1997) and Masters (2002) for more details on the fundamentals and practice of spray drying.

In the production of liquid nutritional products, the o/w emulsion is sterilised. This is achieved by thermal treatments such as UHT processing (e.g. 135–150 °C for 3–5 s) or in-container retort sterilisation (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 dependant on formulation variables such as the amount of protein and the sources used, fat content, pH and ionic strength (McSweeney et al., 2004).

In infant nutritional products, if the emulsion is not sufficiently heat stable, fat globule aggregation occurs 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 and is destroyed upon sterilisation.

8.4 Emulsifying Ingredients in Infant Nutritional Products

The emulsifiers that are used in the production of infant nutritional products 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 nutritional products is outlined in Table 8.2. Bovine milk proteins are widely used in the production of infant nutritional products.

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-lactoglobulins the most abundant whey protein in bovine milk is absent from human milk. This has led to the development of protein fractions enriched in the whey proteins abundant in human milk, particularly α-lactalbumin (Lein, 2003, O'Callaghan & Wallingford, 2002) specially designed for infant formulae. Lactose-free

Table 8.2 Protein ingredients commonly used in infant nutritional products

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 concentrates	Infant formulae
Milk protein isolate	Lactose free infant formulae
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

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 nutritional products contain free amino acids and are devoid of protein or peptides. The non-protein emulsifiers are the sole 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 nutritional products work on the principle that it is prudent to keep the number of additives to the minimum necessary (Scientific Committee for Food, European Commission, 1994). The producers of infant formula take into account the considerable amount of safety studies

Table 8.3 Emulsifiers permitted in infant nutritional products

E. No.	Name	Maximum level	Application
E322	Lecithins	[a]1 g/L	Infant formulae and follow-on formulae
E472c	Mono and diglycerides	[a]4 g/L	Infant formulae and follow-on formulae
		5 g/L	Infant formulae and follow-on formulae for special medical purposes
E471c	Citric acid esters of mono- and diglycerides of fatty acids	[a]9 g/L	Infant formulae and follow-on formulae (in products containing hydrolysed proteins, peptides or amino acids)
E473	Sucrose esters of fatty acids	[a]120 mg/L	Infant formulae and follow-on formulae (in products containing hydrolysed proteins, peptides or amino acids)
E1450	Starch sodium octenyl succinate	20 g/L	Infant formulae and follow-on formulae for special medical purposes

Adapted from Commission of the European Communities (1991, 1999)

[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

and supporting documentation, not to mention the time and cost, required to prove safety of an emulsifier in infant nutritional products. 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-di-glycerides (E471)) 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 stabilise the emulsion. This is reflected in a more extensive list of permitted emulsifiers in these speciality infant nutritional products (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 & Drugs Act, 2003).

Lecithin is widely used as an emulsifier in the food industry. Vegetable-based lecithin is commonly produced as 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 thus, functionality. It 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, for e.g. a phosphatidylcholine enriched lecithin fraction, is possible due to the differences in the solubility of the 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 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% monoglycerides, 38–35% diglycerides, 8–12% triglycerides and 1–7% free glycerol (Moonen & 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 & 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 solubility in warm oils. DATEM is an ionic o/w emulsifier and are more hydrophilic that its constituent mono- and di-glycerides (Gaupp & 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 1–16 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 & Cooper, 2004). These emulsifiers are tasteless, odourless and display a capacity to inhibit microbial growth (Fontecha & 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, and the solution is an opaque suspension (Viswanathan, 1999).

8.5 Stabilising 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.

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 (Codex Alimentarius Commission, 1981) 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 nutritional products 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

Table 8.4 Hydrocolloids permitted in infant nutritional products

E. No.	Name	Maximum level	Application
E412	Guar gum	1 g/L	Infant formulae (where the liquid product contains partially hydrolysed proteins)
		1 g/L	Follow on formula
		10 g/L	From birth onwards in products in liquid formulae containing hydrolysed proteins, peptides or amino acids
E440	Pectins	5 g/L	In acidified follow-on formulae only
		10 g/L	From birth onwards in products used in cases of gastro-intestinal disorders
E407	Carrageenan	0.3 g/L	Follow on formula
E410	Locust bean gum	1 g/L	Follow on formula
		10 g/L	From birth onwards in products for reduction of gastro-oesophageal reflux
E401	Sodium alginate	1 g/L	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 mg/L	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	1.2 g/L	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

Adapted from Commission of the European Communities (1991, 1999)

manufactured to be stable over the shelf-life, which is quite long in the case of infant nutritional products; generally 1–2 years for sterilised liquid emulsions and up to 3 years for powder products. At the end of shelf life the emulsion must have acceptable stability.

Ready-to-feed infant nutritional products are susceptible to similar 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. On shaking, the cream layer may break up into small fat flecks that float on the surface. Alternatively, the fat may form a solid clump, which may prove difficult to re-disperse. A fat ring or 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 whitish and another phase below which is depleted in fat and is generally more translucent in appearance. If the product contains insoluble minerals, a layer of sediment may form over time on the base. 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 dried. Generally, creaming is not an issue as the product is consumed within hours of rehydration but if the emulsion was of a poor quality before drying, undesirable features such as 'oiling off', greasiness and white flecks may become evident after reconstitution.

An understanding of the factors that influence the stability of infant nutritional emulsions is required in order to develop products that display an excellent appearance over a lengthy shelf life.

8.6.2 Emulsifier Functionality

The function of emulsifiers in infant nutritional products is to facilitate the formation of a stable emulsion and to improve stability. This is achieved during the 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 dependant 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 & 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 & Kinsella (1990). Recent aspects of protein-stabilised emulsions were reviewed by McClements (2004). 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).

The emulsifiers commonly used in the production of infant nutritional products are listed in Table 8.5. Regular infant nutritional products can rely on the inherent emulsification properties of intact milk proteins to form stable emulsions. Nutritional products 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 (McClements, 2005, Hasenhuettl, 1997, Faergemand & Krog, 2003). The head group may be non-ionic (e.g. monoglycerides, 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., 1989a).

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 from proteins alone. Consequently, the competitive adsorption of protein 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. 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 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) are more efficient at displacing proteins from the interface than non-ionic oil-soluble emulsifiers are (e.g. monoglycerides) (Dickinson, 1995; Oortwijn & Walstra, 1979; Dickinson & Tanai, 1992, Dickinson et al., 1993a,b,c, Euston et al., 1995). Some non-protein surfactants interact and form complexes with proteins at the interface without necessarily displacing them (Doxastakis & 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 hydro-

Table 8.5 Emulsifying ingredients (both protein and non-protein) and stabilisers used in a selection of some commercially available infant formula

			Emulsifiers used			
Formula type	Brand name	Producer	Protein	Non-protein	Stabiliser	Format
First age and follow-formula based on intact proteins						
Whey dominant	S26	Wyeth Nutrition	Skim milk, Reduced minerals whey	Soy lecithin, mono-di-glycerides	–	Powder, ready-to-feed
Whey dominant	Similac PM 60/40	Ross-Abbot	WPC, sodium caseinate	–	–	Powder
Whey dominant	NAN	Nestle	Reduced minerals WPC, skim milk powder (SMP)	–	–	Powder
Whey dominant	Aptamil Extra	Milupa	Whey powder, skim milk	Soy lecithin	–	Powder
Casein dominant	Similac Advance	Ross-Abbot	Skim milk	Soy lecithin and mono-di-glycerides (in liquids only)	–	Powder, ready-to-feed, concentrate
Formula containing hydrolysed proteins, peptides or amino acids						
First age	S26 HA	Wyeth Nutrition	Partially hydrolysed whey protein	CITREM	–	Powder, ready-to-feed
FSMP	Peptamen	Nestle	Partially hydrolysed whey protein	Soy lecithin	Corn starch, guar gum	powder
First age (lactose-free, soy protein based)	Goodstart 2 Supreme Soy	Nestle	Enzymatically hydrolysed soy protein isolate (SPI)	Soy lecithin	–	powder
Follow-on formula	Goodstart 2	Nestle	Enzymatically hydrolysed Reduced minerals WPC	–	Carrageenan (liquids only)	Powder, ready-to-feed, concentrate
Infant formula (based on free amino acids)	Neocate	SHS	(amino acid-based)	CITREM	–	
Medical food for children ages 1–10	Neocate Junior	SHS	(amino acid-based)	DATEM	Propylene Glycol Alginate	powder

(continued)

Table 8.5 (continued)

Formula type	Brand name	Producer	Emulsifiers used		Stabiliser	Format
			Protein	Non-protein		
Medical food for children ages 1–10	Neocate One+	SHS	(amino acid-based)	mono-di-glycerides, DATEM	Propylene Glycol Alginate	powder
Medical food for children ages 1–10	Pediatric E028	SHS	(amino acid-based)	mono-di-glycerides, DATEM	Sodium Carboxy methyl cellulose	ready-to-feed
Medical food, based on free amino acids and non-dairy hydrolysates, for children ages 1–10	Pepdite One+	SHS	Hydrolyzed pork and soy Proteins (and free amino acids)	DATEM	–	powder
First age, whey dominant	Enfamil Gentlease LIPIL	Mead Johnson	Partially hydrolysed skim milk and whey protein solids	mono-di-glycerides, DATEM	Propylene Glycol Alginate	powder
	Enfamil Pregestimil LIPIL	Mead Johnson	Casein hydrolysate	Acetylated monoglycerides N(powder only)	Starch in powder Starch and carrageenan in liquids	Powder, ready-to-feed, concentrate
First age	Similac Alimentum Advance	Ross-Abbot	Casein hydrolysates (supplemented with free amino acids)	DATEM (powder only)	Xanthan gum in powder, Starch and carrageenan in liquids	ready-to-feed, powder
	Nutramigen LIPIL	Mead Johnson	extensively hydrolyzed casein (supplemented with free amino acids)	Acetylated monoglycerides	Starch in powder Starch and carrageenan in liquids	Powder, ready-to-feed, concentrate

phobic interactions (Dickinson, 1993). Several studies have demonstrated that surfactants interact with dairy proteins (Brown et al., 1982, 1983; Fontecha & Swaisgood, 1994, 1995; Sarker et al., 1995; Antipova et al., 2001; Deep & 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-glycerides affect fat crystallisation and crystal structure in emulsion droplets (Euston, 1997), which may destabilise the o/w emulsions (Boode & 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. As already mentioned, the influence of surfactants on heat stability is of particular relevance to the manufacture of heat-sterilised recombined milk based beverages such as ready to feed infant formulae.

8.6.2.1 Functional Properties of Proteins as Emulsifiers

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 (Atkinson et al., 1995; Brooksbank et al., 1993; Courthaudon et al., 1991; Dalgleish, 1993; Dickinson et al., 1993a,b; Dickinson et al., 1988; Leermakers et al., 1996; Leaver & Dalgleish, 1992). Similarly, the emulsifying characteristics of the individual whey proteins, including ß-lactoglobulin (β-lg), α-lac and bovine serum albumin (BSA) have been studied (Atkinson et al., 1995; Dickinson & Gelin, 1992; Eaglesham et al., 1992; Dickinson & Matsumura, 1991, 1994; Dickinson & Iveson, 1993; Dickinson et al., 1993). 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 more slowly 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 & Walstra, 1979, 1982; Britten & Giroux, 1991; Sharma & Dalgleish, 1993; Sharma & Singh 1998; Brun & 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 nutritional products (Britten & Giroux, 1991; Sharma & Singh, 1998; Euston & Hirst, 1999, 2000; Sourdet et al., 2002; McSweeney et al., 2004).

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 than 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 form emulsions 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 & 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-stabilised emulsions but had no effect on sodium caseinate stabilised 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 as milk protein concentrate (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-stabilised 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 leading to reduced creaming stability (Euston & 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).

Those infant nutritional products, 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 studied (Britten & 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 destabilisation 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 spray drying and reconstitution emulsions (20%, w/w, soybean oil; 2.4% protein) prepared from SMP alone, WPI alone or SMP/WPI blends had little impact on the amount of adsorbed protein. Characterisation of the interfacial proteins showed that the composition of the adsorbed layer of casein-dominant emulsions was largely unaffected by spray drying and reconstitution. However, 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) enriched whey protein fractions, have been developed especially for use in infant nutritional products. These fractionated ingredients may be less efficient emulsifiers than 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 & Singh, 1998).

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 or whey protein and the stability of resultant emulsions. Some studies have reported that the emulsion forming ability of low DH hydrolysates of casein (Chobert et al., 1988a,b; Haque & Mozaffar, 1992) or whey protein (Haque & Mozaffar, 1992; Vojdani & 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 & Fitzgerald, 1998; Euston et al., 2001b). In general, intact milk proteins form more stable emulsions than hydrolysates of milk proteins (Haque & Mozaffar, 1992; Agboola & 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 non-hydrolyzed 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 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 stabilised against creaming and coalescence, when a low level of protein was used in combination with hydrolysed lecithin and glucose syrup.

Emulsifying Properties of Soy Protein Sources

Non-dairy infant nutritional products 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 are hence can act as emulsifiers. Mitidieri and Wagner (2002) and Palazolo et al. (2003) found that oil-in-water emulsions, stabilised using native SPI (at concentrations in the range 1–10 mg ml^{-1}) were very stable against coalescence but emulsions prepared with denatured SPI were unstable. These results were linked to the 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 multiplayer film that was susceptible to stress.

8.6.2.2 Functional Properties of Non-Protein Emulsifiers

Lecithin

As lecithin has intermediate solubility characteristics and HLB numbers (~8), it is not particularly suitable for stabilising 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 nutritional products.

The main surface-active components of lecithin, the phopholipids (PC, PE, PI and PA) consist 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 functional at ambient temperatures unlike the other widely used emulsifier in infant nutritional products, the mono-di-glycerides, which must be melted at ~70 °C to function.

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 & Araki, 1997) and oil droplet size (Dickinson & 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

& Szuhaj, 1998; Rydhag & Wilton, 1981). The emulsion stabilising effect of zwitterionic 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 & 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 phopholipid/protein interactions (Dickinson, 1997). In general, phospholipids are not very effective at completely displacing milk proteins from the o/w interface (Dickinson & Iveson, 1993; Fang & Dalgleish, 1996a,b). For example, Courthaudon et al. (1991a) 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 & Dalgleish, 1993). Several studies have demonstrated an interaction of certain phospholipids with milk proteins in general (Korver & Meder, 1974) or 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., 1991a; Dickinson et al., 1993a) and the formation of protein/phospholipid complexes (Kristensen et al., 1997; Lefèvre & 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. One of the reasons for using lecithin in ready-to-feed infant nutritional products is to increase heat stability (McSweeny et al., in press). Several studies have demonstrated that lecithin improves the heat stability of milk (Hardy et al., 1985; McCrae & Muir, 1992; Singh et al., 1992), whey protein stabilised 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.

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 destabilised. 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 organised structure at the interface.

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, monoglycerides 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 nutritional products.

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 & Larsson, 1992; Gelin et al., 1994; Pelan et al., 1997; Davies et al., 2000, 2001).

- 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^{-1} in the oil phase, saturated monoglycerides (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 monoglycerides may be able to align in more closely packed layers at the interface compared to the fatty acid chains of unsaturated monoglycerides.

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). In particular, mono-di-glycerides are very effective at displacing proteins from the interface at temperatures below ~15 °C. Upon cooling, mono-di-glycerides promote fat crystallisation; 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 monoglycerides (GMS, GMP) have a greater ability to initiate fat crystallisation than unsaturated

monoglycerides such as GMO (Davies et al., 2001). The presence of fat crystals further promotes the destabilisation 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 crystallisation during the storage of infant nutritional emulsions 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 promoted the heat-induced aggregation of a whey protein stabilised emulsion.

Organic Esters of Mono-Di-Glycerides (Citrem and Datem)

CITREM (E472c) and DATEM (E472d) are used in the production of infant nutritional products 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 stabilising the emulsion through electrostatic repulsion. The electrostatic repulsion prevents coalescence and thus products with reasonably long shelf lives can be produced.

Organic esters of mono-di-glycerides are widely used in the baking industry and there is not so much information in the literature on how these ingredients behave in fluid o/w emulsions. Antipova et al. (2001) demonstrated that like other surfactants, CITREM interacts with aqueous phase proteins, in this case sodium caseinate, predominantly through hydrophobic interactions. CITREM was demonstrated to be an extremely effective emulsifier in stabilizing a model ready-to-feel infant formula emulsion containing hydrolysed whey protein; emulsions made using CITREM as the only added emulsifier had small fat globules (<1μm) and demonstrated stability towards Geaming, coalescence and retort sterilization (McSweeny, 2007). Giroux and Britten (2004) demonstrated that DATEM interacts with whey proteins to modify their structure and thermal stability and but not as extensively as sodium dodecyl sulphate (SDS) or sodium stearoyl-2 lactylate (SSL).

Sucrose Esters of Fatty Acids

Sucrose esters of fatty acids (E473) may be used in the production of infant formula 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 nutritional products (Table 8.5). There is a lack of information on the literature related to the use of sucrose esters of fatty acids in fluid o/w emulsions.

Starch Octenyl Succinate Anhydride

When the starch octenyl succinate anhydride (OSA starch) macromolecule adsorbs at the o/w interface it stabilises droplets against coalescence by steric hindrance and charge repulsion. 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 stabilised emulsions were not susceptible to aggregation near the iso-electric point of the protein. Mahmoud (1987) reported that OSA starch was very effective in stabilising a hypoallergenic formula based on extensively hydrolysed proteins. Although a permitted ingredient in certain circumstances, OSA starch (E1450) is not a widely used ingredient in the production of infant nutritional products (Table 8.5).

8.6.3 Function of Stabilisers

Traditionally, hydrocolloids such as gums and starches have been regarded as thickeners. Their stabilising 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.

8.7 Summary

Infant nutritional products 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 nutritional products that are based on intact proteins may be stabilised 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. In addition to lecithin and mono-di-glycerides, other emulsifiers (CITREM, DATEM, OSA starch and sucrose esters of fatty acids) and stabilisers are permitted for use in infant nutritional products that are based on hydrolysed proteins, peptides or amino acids. Apart from the emulsifiers used, the emulsion quality of infant nutritional products is influenced by other compositional variables; protein-stabilised emulsions are especially sensitive to pH and ionic strength effects (McClements, 2004). Therefore, infant nutritional products 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 dependant on the conditions during the homogenisation step (method, temperatures, pressure, number of passes)

and unit operations that thermally process the emulsion such as the terminal sterilisation 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.

References

Agboola, S.O. & Dalgleish, D.G. (1996). Enzymatic hydrolysis of milk proteins used for emulsion formation. 1. Kinetics of protein breakdown and storage stability of the emulsions. *J. Agric. Food Chem*., **44**, 3631–3636.

Antipova, A.S., Semenova, M.G., Belyakova, L.E. & Il'in, M.M. (2001). On relationships between molecular structure, interaction and surface behaviour in mixture: small-molecule surfactant + protein. *Colloids Surf. B Biointerfaces*, **21**, 217–230.

Aoki, H., Taneyama, O. & Inami M. (1980). Emulsifying properties of soy proteins: Characteristics of 7S and 11S proteins. *J. Food Sci.,* **45**, 534–538.

Arts, T.J.C., Laven, J., Voorst Vader, F. van & Kwaaitaal, T. (1994). Zeta potentials of tristearoylglycerol crystals in olive oil. *Colloid Surf. A, Physicochem. Eng. Aspects*, **85**, 149–158.

Atkinson, P.J., Dickinson, E. Horne, D.S. & Richardson R.M. (1995). Neutron reflectivity of adsorbed β-casein and β-lactoglobulin at the air/water interface. *J. Chem. Soc. Faraday Trans*., **91**, 2847–2854.

Barfod, N.M., Krog, N. Larsen, G. & Buchheim, W. (1991). Effects of emulsifiers on protein/fat interaction in ice-cream mix during ageing. 1. Quantitative analyses. *Fat Sci. Technol*., **93**, 24–29.

Boode, K. & Walstra, P. (1993). Kinetics of partial coalescence in oil-in-water emulsions. In, *Food Colloids and Polymers: Stability and Mechanical Properties* (eds. E. Dickinson & P. Walstra), Royal Soc. Chem., Cambridge, UK, pp. 23–30.

Boyd, J.V., Mitchell, J.R., Irons, L., Musselwhite, P.R & Sherman, P. (1973). The mechanical properties of milk protein films spread at the air-water interface. *J. Colloid Interface Sci*., **45**, 478–486.

Britten, M. & Giroux, H.J. (1991). Emulsifying properties of whey protein and casein composite blends. *J. Dairy Sci.,* **74**, 3318–3325.

Brooksbank, D.V., Davidson, C.M., Horne, D.S. & Leaver, J. (1993). Influence of electrostatic interactions on β-casein layers adsorbed on polystyrene lattices. *J. Chem. Soc. Faraday Trans*., **89**, 3419–3425.

Brown, E.M., Carroll, R.J., Pfeffer, P.E. & Sampugna, J. (1983). Complex formation in sonicated mixtures of β-lactoglobulin and phosphatidylcholine. *Lipids*, **18**, 111–118.

Brown, E.M., Sampugna, J., Pfeffer, P.E. & Carroll, R.J. (1982). Interaction of phosphatidylcholine with beta-lactoglobulin. *Biophys. J.,* **37**, 71–72.

Brun, J.M. & Dalgleish, D.G. (1999). Some effects of heat on the competitive adsorption of caseins and whey proteins in oil-in-water emulsions. *Int. Dairy J*., **9**, 323–327.

Bueschelberger, H-.G. (2004). Lecithins. In, *Emulsifiers in Food Technology* (ed. Robert J. Whitehurst), Blackwell Publishing Ltd., Oxford, UK, pp. 1–39.

Canadian Food & Drugs Act (2003). Part B. Food and Drug Regulations. Division 24. Foods for special dietary use. Department of Health, Ottawa, Ontario, Canada.

Carver, J.D., Wu, P.Y., Hall, R.T., Ziegler, E.E., Sosa, R., Jacobs, J., Baggs, G., Auestad, N. & Lloyd, B. (2001). Growth of preterm infants fed nutrient-enriched or term formula after hospital discharge. *Paediatrics*, **107**, 683–689.

Chobert, J.M., Bertrand-Harb, C. & Nicolas, M.G. (1988a). Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *J. Agric. Food Chem.,* **36**, 883–892.

Chobert, J.M., Sitohy, M.Z. & Whitaker, J.R. (1988b). Solubility and emulsifying properties of caseins modified enzymatically by Staphylococcus aureus V8 protease. *J. Agric. Food Chem*., **36**, 220–224.

Codex Alimentarius Commission (1981). Codex standard for infant formula, Codex Stan. 72–1981, as amended in 1983, 1985, 1987 and 1997. Food and Agriculture Organization of the United Nations and World Health Organization, Rome, Italy.

Commission of the European Communities (1991). Commission directive of 14 May 1991 on infant formulae and follow-on formulae. 92/321/EEC. (Last consolidated 25 May 1999). *Official J. Eur. Comm*., **L175**, 35–49.

Commission of the European Communities (1999). Commission directive of 25 March 1999 on Dietary Foods for Special Medical Purposes. 1999/21/EC. *Official J. Eur. Comm*., **L91/29**, 29–36.

Courthaudon, J.-L., Dickinson, E. & Christie, W.W. (1991). Competitive adsorption of lecithin and β-casein in oil-in-water emulsions. *J. Agric. Food Chem*., **39**, 1365–1368.

Courthaudon, J.-L., Girardet, J.M., Campagne, S., Rouhier, L.M. & Campagne, S., Linden, G. & Lorient, D. (1999). Surface active and emulsifying properties of casein micelles compared to those of sodium caseinate. *Int. Dairy J*., **9**, 411–412.

Dalgleish, D.G. (1993). The sizes and conformations of the proteins in adsorbed layers of individual caseins on lattices and in oil-in-water emulsions. *Colloids Surf. B. Biointerfaces,* **1,** 1–8.

Dalgleish, D.G. (1996). Food emulsions. In, *Emulsions and Emulsion Stability* (ed. J. Sjöblom), Marcel Dekker Inc, New York, pp. 287–321.

Dalgleish, D.G., Goff, H.D., Brun, J.M. & Luan, B. (2002). Exchange reactions between whey proteins and caseins in heated soya oil-in-water emulsion systems—overall aspects of the reaction. *Food Hydrocolloids*, **16**, 303–311.

Das, K.P. & Kinsella, J.E. (1990). Stability of food emulsions: Physicochemical role of protein and nonprotein emulsifiers. *Adv. Food Nutr. Res.,* **34**, 81–129.

Davies, E., Dickinson, E. & Bee, R.D. (2000). Shear stability of sodium caseinate emulsions containing monoglyceride and triglyceride crystals. *Food Hydrocolloids*, **14**, 145–153.

Davies, E., Dickinson, E. & Bee, R.D. (2001). Orthokinetic destabilization of emulsions by saturated and unsaturated monoglycerides. *Int. Dairy J*., **11**, 827–836.

Deep, S. & Ahluwalia, J.C. (2001). Interaction of bovine serum albumin with anionic surfactants. *Phys. Chem. Chem. Phys*., **3**, 4583–4591.

de Feijter, J.A., Benjamins, J. & Tamboer, M. (1987). Adsorption displacement of proteins by surfactants in oil-in-water emulsions. *Colloids Surf*., **27**, 243–266.

Dickinson, E. (1993). Proteins in solution and at interfaces. In, *Interactions of Surfactants with Polymers and Proteins* (eds. E.D. Goddard & K.P. Ananthapadmanabhan), CRC Press, Boca Raton, FL., p 295.

Dickinson, E. (1995). Recent trends in food colloids research. In, *Food Macromolecules and Colloids* (eds. E. Dickinson & D. Lorient), Royal Soc. Chem., Cambridge, United Kingdom, p 1.

Dickinson, E. (1997). Properties of emulsions stabilized with milk proteins: overview of some recent developments. *J. Dairy Sci*., **80**, 2607–2619.

Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids Surf. B. Biointerfaces,* **20**, 197–210.

Dickinson, E. (2004). Properties of emulsions stabilized with milk proteins: Overview of some recent developments. *J. Dairy Sci.,* **80**, 2607–2619.

Dickinson, E., & Gelin, J.-L. (1992). Influence of emulsifier on competitive adsorption of α_s-casein and β-lactoglobulin in oil-in-water emulsions. *Colloids Surf. B. Biointerfaces,* **63,** 329–335.

Dickinson, E., Golding, M. &. Povey, M.J.W. (1997). Creaming and flocculation of oil-in-water emulsions containing sodium caseinate. *J. Colloid Interface Sci*., **185**, 515–529.

Dickinson, E., Horne, D.S. & Richardson, R.M. (1993a). Neutron reflectivity study of the competitive adsorption of β-casein and water-soluble surfactant at the planar air-water interface. *Food Hydrocolloids*, **7**, 497–505.

Dickinson, E. & Iveson, G. (1993). Absorbed films of β-lactoglobulin and lecithin at the hydrocarbon-water and triglyceride-water interfaces. *Food Hydrocolloids,* **6**, 533–541.

Dickinson, E. & Matsumura, Y. (1991). Time-dependent polymerisation of b-lactoglobulin through disulphide bonds at the oil-water interface in emulsions. *Int. J. Biol. Macromol*., **13**, 26–30.

Dickinson, E. & Matsumura, Y. (1994). Proteins at liquid interfaces: Role of the molten globule state. *Colloids Surf. B. Biointerfaces,* **3,** 1–17.

Dickinson, E., Mauffret, A. Rolfe, S.E & Woskett, C.M. (1989a). Adsorption at interfaces in dairy systems. *J. Soc. Dairy Technol*., **42**, 18–22.

Dickinson, E., Narhan, S.K. & Stainsby, G. (1989b). Stability of cream liqueurs containing low-molecular-weight surfactants. *J. Food Sci.,* **54**, 77–81.

Dickinson, E., Owusu, R.K., Tan, S. & Williams, A. (1993b). Oil-soluble surfactants have little effect on competitive adsorption of alpha-lactalbumin and beta-lactoglobulin in emulsions, *J. Food Sci*., **58**, 295–298.

Dickinson, E., Owusu, R.K. & Williams, A. (1993c). Orthokinetic destabilization of a protein-stabilized emulsion by a water-soluble surfactant. *J. Chem. Soc. Faraday Trans*., **89**, 865–66.

Dickinson, E., Rolfe, S.E. & Dalgleish, D.G. (1988). Competitive adsorption of α_{s1}-casein and β-casein in oil-in-water emulsions. *Food Hydrocolloids*, **2**, 397–405.

Dickinson, E. & Tanai, S. (1992). Protein displacement from the emulsion droplet surface by oil-soluble and water-soluble surfactants. *J. Agric. Food Chem.,* **40**, 179–183.

Doxastakis G. & Sherman, P. (1984). The interaction of sodium caseinate with monoglyceride and diglyceride at the oil-water interface and its effect on interfacial rheological properties. *Colloid Polym. Sci.,* **264**, 254–259.

Eaglesham, A., Herrington, T.M. & Penfold, J. (1992). A neutron reflectivity study of a spread monolayer of bovine serum albumin. *Colloids Surf*., **65**, 9.

Euston, S.E., Singh, H., Munro, P.A. & Dalgleish, D.G. (1995). Competitive adsorption between sodium caseinate and oil-soluble and water-soluble surfactants in oil-in-water emulsions. *J. Food Sci*., **60**, 1124–1131.

Euston, S.R. (1997). Emulsifiers in Dairy Products and Dairy Substitutes. In, *Food Emulsifiers and their Applications* (eds. G.L. Hassenheutl & R. Hartel), Chapman & Hall, New York, pp. 173–210.

Euston, S.R. & Hirst, R.L. (1999). Comparison of the concentration-dependent emulsifying properties of protein products containing aggregated and non-aggregated milk protein. *Int. Dairy J*., **9**, 693–701.

Euston, S.R. & Hirst, R.L. (2000). The emulsifying properties of commercial milk protein products in simple oil-in-water emulsions and in a model food system. *J. Food Sci*., **65**, 934–940.

Euston, S.R., Finnigan, S.R. & Hirst, R.L. (2001a). Aggregation kinetics of heated whey protein-stabilized emulsions: effect of low-molecular weight emulsifiers. *Food Hydrocolloids,* **15**, 253–262.

Euston, S.R., Finnigan, S.R. & Hirst, R.L. (2001b). Heat-induced destabilization of oil-in-water emulsions formed from hydrolyzed whey protein. *J. Agric. Food Chem*., **49**, 5576–5583.

Faergemand, M. & Krog, N. (2003). Using emulsifiers to improve food texture. In, *Texture in Foods, Volume 1: Semi-Solid Foods* (ed. B.M. McKenna), CRC Press, Boca Raton, FL. Chapter 10.

Fang, Y., & Dalgleish, D.G. (1993). Casein adsorption on the surfaces of oil-in-water emulsions modified by lecithin. *Colloids Surf*., **1,** 357–364.

Fang, Y. & Dalgleish, D.G. (1996a). Comparative effects of three different phosphatidylcholines on casein-stabilized oil-in-water emulsions. *J. Am. Oil Chem. Soc.,* **73**, 437–442.

Fang, Y. & Dalgleish, D.G. (1996b). Competitive adsorption between dioleoylphosphatidylcholine and sodium caseinate on oil-water interfaces. *J. Agric. Food Chem*., **44**, 59–64.

Fontecha, J. & Swaisgood, H. (1995). Interaction of sucrose esters with skim milk proteins as characterised by size-exclusion chromatography. *J. Dairy Sci*., **78**, 2660–2665.

Fontecha, J. & Swaisgood, H. (1994). Interaction of sucrose esters with skim milk proteins as characterised by affinity chromatography. *J. Dairy Sci*., **77**, 3545–3551.

Friberg, S.E. & Solans, C. (1986). Surfactant association structures and the stability of emulsions and foams. *Langmuir*, **2**, 121–126.

FSANZ (2000). *Australia and New Zealand Food Standards Code. Standard 2.9.1. Infant formula products*. FSANZ, Canberra, Australia.

Gaupp, R. & Adams, W. (2004). Acid esters of mono- and diglycerides. In, *Emulsifiers in Food Technology* (ed. Robert J. Whitehurst), Blackwell Publishing Ltd., pp. 59–85.

Gelin, J.-L., Poyen, L. Courthaudon, J.-L. Le Meste, M. & Lorient, D. (1994). Structural changes in oil-in-water emulsions during the manufacture of ice cream. *Food Hydrocolloids*, **8**, 299–308.

Giroux, H.J. & Britten, M. (2004). Heat treatment of whey proteins in the presence of anionic surfactants. *Food Hydrocolloids*, **18**, 685–692.

Haque, Z.U. & Mozaffar, Z. (1992). Casein hydrolysate. II. Functional properties of peptides. *Food Hydrocolloids*, **5**, 559–571.

Hardy, E.E., Sweetsur, A.W.M., West, I.G. & Muir, D.D. (1985). Heat stability of concentrated milk: enhancement of initial heat stability by incorporation of food grade lecithin. *Aust. J. Food Technol.,* **20**, 97–105.

Hasenhuettl, G.L. (1997). Overview of food emulsifiers. In, *Food Emulsifiers and Their Applications* (eds. G.L. Hasenhuettl & R.W. Hartel), Chapman & Hall, New York, NY.

Istarova, T.A., Semenova, M.G., Sorokoumova, G.M., Selishcheva, A.A., Belyakova, L.E., Polikarpov, Y.N. & Anokhina, M.S. (2005). Effect of pH on the interactions of sodium caseinate with soy phospholipids in relation to the foaming ability of their mixtures. *Food Hydrocolloids*, **19**, 429–440.

Jimenez-Flores, R., Ye, A. & Singh, H. (2005). Interactions of whey proteins during heat treatment of oil-in-water emulsions formed with whey protein isolate and hydroxylated lecithin. *J. Agric. Food Chem.,* **53**, 4213–4219.

Kieseker, F.G. (1983). Recombined dairy products. *CSIRO Food Res. Q*., **43**, 25–37.

Korver, O. & Meder, H. (1974). The influence of lysolecithin on the complex formation between beta-lactoglobulin and kappa-casein. *J. Dairy Res.,* **41**, 9–17.

Kristensen, A., Nylander T., Paulsson, M. & Carlsson, A. (1997). Calorimetric studies of interactions between β-lactoglobulin and phospholipids in solution. *Int. Dairy J.,* **7**, 87–92.

Krog, N. & Larsson, K. (1992). Crystallization at interfaces in food emulsions—A general phenomenon. *Fat Sci. Technol*., **94**, 55–57.

Lajoie, N., Gauthier, S.F. & Pouliot, Y. (2001). Improved storage stability of model infant formula by whey peptides fractions. *J. Agric. Food Chem*., **49**, 1999–2007.

Leaver, J. & Dalgleish, D.G. (1992). Variations in the binding of β–casein to oil-water interfaces detected by trypsin-catalysed hydrolysis. *J. Colloid Interface Sci*., **149**, 49–55.

Leermakers, F.A.M., Atkinson, P.J., Dickinson, E. & Horne, D.S. (1996). Self-consistent-field modelling of adsorbed β-casein: effects of pH and ionic strength on surface coverage and density profile. *J. Colloid Interface Sci*., **178**, 681–693.

Lefèvre, T. & Subirade, M. (2001). Molecular structure and interaction of biopolymers as viewed by Fourier transform infrared spectroscopy: Model studies on β-lactoglobulin. *Food Hydrocolloids*, **15**, 365–376.

Lein, E. (2003). Infant formulae with increased concentrations of α-lactalbumin. *Am. J. Clin. Nutr. (suppl.),* **77**, 1555S–1558S.

Lucas, A., Fewtrell, M.S., Morley, R. et al. (2001). Randomized trial of nutrient-enriched formula versus standard formula for post discharge preterm infants. *Paediatrics*, **108**, 703–711.

Mahmoud, M.I. (1987). Enteral nutritional hypoallergenic formula. *US Patent 4,670,268*.

Master, K. (2002). *Spray Drying inPractice*. SprayDryConsult International ApS, Charlottenlund, Denmark Publishers.

McClements, D.J. (2004). Protein stabilized emulsions. *Curr. Opin. Colloid Interface Sci*., **9**, 305–313.

McClements, D.J. (2005). *Food Emulsions: Principles, Practice and Techniques*—2nd edition. (ed. D.J. McClements), CRC Press, Boca Raton, FL, Chapter 4.

McCrae, C.H. & Muir, D.D. (1992). The influence of phospholipid classes of crude lecithin on the heat stability of recombined milk. *Milchwissenschaft*, **47**, 755–759.

McSweeney S.L., Mulvihill, D.M. & O'Callaghan, D.M. (2004). The influence of pH on the heat-induced aggregation of model milk protein ingredient systems and model infant formula emulsions stabilized by milk protein ingredients. *Food Hydrocolloids*, **18**, 109–125.

McSweeney, S.L. (2007). Stability of model ready-to feed infant formula emulsions. Ph. D. Thesis, National University of Ireland, Cork.

McSweeney, S.L., Healy, R. & Mulvihill, D.M. (in press). Effect of lecithin and monoglycerides on the heat stability of a model infant formula emulsion. Food Hydrocolloids, Available online 7 May 2007.

Mellema, M. & Isenbart, J.G. (2004). Effect of acidification and heating on the rheological properties of oil-water interfaces with adsorbed milk proteins. *J. Dairy Sci*., **87**, 2769–2778.

Mitidieri, F.E. & Wagner, J.R. (2002). Coalescence of o/w emulsions stabilized by whey and isolate soybean proteins. Influence of thermal denaturation, salt addition and competitive interfacial adsorption. *Food Res. Int*., **35**, 547–557.

Miura, S., Yamamoto, A. & Sato, K. (2002). Effect of monoacylglycerols on the stability of model cream using palm oil. *Eur. J. Lipid Sci. Technol*., **104**, 819–824.

Moonen, H. & Bas, H. (2004). Mono- and diglycerides. In, *Emulsifiers in Food Technology* (ed. R.J. Whitehurst). Blackwell Publishing Ltd., Oxford, UK, pp. 40–58.

Mulvihill, D.M. & Murphy, P.C. (1991). Surface active and emulsifying properties of caseins/caseinates as influenced by state of aggregation. *Int. Dairy J*., **1**, 13–37.

Nelen, B.A.P. & Cooper, J.M. (2004). Sucrose esters. In, *Emulsifiers in Food Technology* (ed. R. J.Whitehurst), Blackwell Publishing Ltd., Oxford, UK, pp. 131–158.

O'Callaghan, D.M. & Wallingford, J.C. (2002). Infant formulae—New developments. In, *Encyclopaedia of Dairy Science* (eds. H. Roginshki, J. Fuquay & P.F. Fox), Academic Press, Elsevier Science, London., Vol. 3, pp. 1384–1392.

Olson, D.W., White, C.H. & Richter R.L. (2004). Effect of pressure and fat content on particle sizes in microfluidized milk. *J. Dairy Sci.,* **87**, 3217–3223.

Oortwijn, H., & Walstra, P. (1979). Membranes of recombined fat globules. 2. Composition. *Neth. Milk Dairy J*., **33**, 134–154.

Oortwijn, H. & Walstra, P. (1982). Membranes of recombined fat globules. 4. Effects on properties of recombined milks. *Neth. Milk Dairy J*., **36**, 279–290.

Palazolo, G.G., Mitidieri, F.E. & Wagner, J.R. (2003). Relationship between interfacial behaviour of native and denatured soybean isolates and microstructure and coalescence of oil-in-water emulsions—Effect of salt and protein concentration. *Food Sci. Tech. Int*., **9**, 409–11.

Pelan, B.M.C., Watts, K.M., Campbell, I.J. & Lips, A. (1997). On the stability of aerated milk protein emulsions in the presence of small-molecule surfactants. In, *Food Colloids: Proteins, Lipids and Polysaccharides* (eds. E. Dickinson & B. Bergenstahl), Royal Soc. Chem., Cambridge, United Kingdom, p 55.

Pisecky (1997). *Handbook of Milk Powder Manufacture*. Niro A/S Copenhagen, Denmark.

Rahali, V., Chobert, J.M., Haertle, T. & Gueguen, J. (2000). Emulsification of chemical and enzymatic hydrolysates of β-lactoglobulin: characterization of the peptides adsorbed at the interface. *Nahrung*, **44**, 89–95.

Rydhag, L. & Wilton, I. (1981). The function of phospholipids of soybean lecithin in emulsions. *J. Am. Oil Chem. Soc.,* **58**, 830–837.

Sarker, D.K., Wilde, P.J. & Clark, D.C. 1995. Control of surfactant-induced destabilization of foams through polyphenol-mediated protein-protein interactions. *J. Agric. Food Chem.,* **43**, 295–300.

Scientific Committee for Food, European Commission (1994). Opinion on certain additives for use in infant formulae, follow-on formulae and weaning foods. Reports of the Scientific Committee for Food (32nd series). *Office for Official Publications of the European Communities*, Luxembourg.

Sharma, R. & Dalgleish, D.G. (1993). Interactions between milk serum proteins and synthetic fat globule membrane during heating of homogenized whole milk. *J. Agric. Food Chem*., **41**, 1407–1412.

Sharma, R. & Singh, H. (1998). Adsorption behaviour of commercial milk protein and milk powder products in low-fat emulsions. *Milchwissenschaft*, **53**, 373–377.

Singh, H., Sharma, R. & Tokley, R.P. (1992). Influence of incorporation of soya lecithin into skim milk powder on the heat stability of recombined evaporated milk. *Aust. J. Dairy Technol*., **47**, 33–37.

Sjollema, A. (1987). Recombination of milk and dairy ingredients into milk, cream, condensed milk and evaporated milk. In, *Milk—The Vital Force*, Reidel Publishing, Boston, MA, pp. 251–257.

Slattery, H. & Fitzgerald, R.J. (1998). Functional properties and bitterness of sodium caseinate hydrolysates prepared with a Bacillus proteinase. *J. Food Sci*., **63**, 418–422.

Sliwinski, E.L., Lavrijsen, B.W.M., Vollenbroek, J.M., van der Stege, H.J., van Boekel, M.A.J.S. & Wouters, J.T.M. (2003). Effects of spray drying on physicochemical properties of milk protein-stabilised emulsions. *J. Colloids Surf. B Biointerfaces,* **31**, 219–229.

Sourdet, S., Relkin, P., Fosseux, P.Y. & Aubry, V. (2002). Composition of fat protein layer in complex food emulsions at various weight ratios of casein-to-whey proteins. *Lait*, **82**, 567–578.

Srinivasan, M., Singh, H. & Munro, P.A. (1996). Sodium caseinate-stabilized emulsions: Factors affecting coverage and composition of surface proteins. *J. Agric. Food Chem*., **44**, 3807–3811.

Stauffer C.E. (1999). *Emulsifiers*, Eagan Press Handbook, St. Paul, MA, pp. 551–553.

Sunder, A., Scherze, I. & Muschiolik, G. (2001). Physico-chemical characteristics of oil-in-water emulsions based ion whey protein-phospholipid mixtures. *Colloids Surf. B Biointerfaces*, **21**, 75–85.

Tesch, S., Gerhards, C. & Schubert H. (2002). Stabilization of emulsions by OSA starches. *J. Food Eng*., **54**, 167–174.

Tirok, S., Scherze, I. & Muschiolik, G. (2001). Behaviour of formula emulsions containing hydrolysed whey protein and various lecithins. *Colloids Surf. B Biointerfaces*, **21**, 149–162.

Van der Meeren, P., El-Bakry, M., Neirynck, N. & Noppe, P. (2005). Influence of hydrolyzed lecithin on protein adsorption and heat stability of a sterilised coffee cream simulant. *Int. Dairy J*., **15**, 1235–1243.

Van der Ven, C., Gruppen, H., de Bont, D.B.A. & Voragen, A.G.J. (2001). Emulsion properties of casein and whey protein hydrolysates and the relation with other hydrolysate characteristics. *J. Agric. Food Chem*., **49**, 5005–5012.

van Niewenhuyzen, W. & Szuhaj, B.F. (1998). Effects of lecithins and proteins on the stability of emulsions. *Fett/Lipid*, **100**, 282–291.

Viswanathan, A. (1999). Effect of degree of substitution of octenyl Succinate starch on the emulsification activity on different oil phases. *J. Polym. Environ*., **7**, 191–196

Vojdani, F. & Whitaker, J.R. (1994). Chemical and enzymatic modification of proteins for improved functionality. In, *Protein functionality in food systems* (eds. N.S. Hettiarachy & G.R. Ziegler), Marcel Dekker, New York, pp. 261–310.

Walstra, P., Guerts, T.J., Noomen, A. & van Boekel, M.A.J.S. (1999). *Dairy Technology*. Marcel Dekker, New York.

Yamamoto, Y. & Araki, M. (1997). Effects of lecithin addition in oil or water phase on the stability of emulsions made with whey proteins. *Biosci. Biotechnol. Biochem.,* **61**, 1791–1795.

Yamauchi, K., Shimizu, M. & Kamiya, T. (1980). Emulsifying properties of whey protein. *J. Food Sci.,* **45**, 1237–1242.

Zadow, J.G. (1982). Recombined milks and creams. *Int. Dairy Fed. Bull*., **142**, 33–46.

Zielinski, R.J. (1997). Synthesis and composition of food-grade emulsifiers. In, *Food Emulsifiers and their Application*, (eds. G.L. Hasenhuettl & R.W. Hartel), Chapman & Hall, New York, NY, pp. 11–38.