

Infant Formulae: Ingredient Selection, Manufacturing Technology, Innovation, Challenges and Opportunities

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1.1. Introduction

1.1.1. Context for formula-feeding

The Food and Agriculture Organization/World Health Organization (FAO/WHO) give the following definition for infant formula: “Infant formula means a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life, up to the introduction of appropriate complementary feeding” (Joint WHO/FAO/UNU Expert Consultation 2007). In response, infant formulae are primarily designed to mimic the nutritional profile of human milk. While breast-feeding is exclusively recommended for the first six months of life, infant formulae provide a suitable alternative in specific situations where the mother cannot or does not want to breast-feed (Guo 2014). Indeed, the WHO advises that mothers of new-born babies should be informed of the benefits and superiority of breast-feeding. However, formula-feeding presents advantages, such as meeting specific nutritional requirements, convenience and flexibility. If formula-feeding is chosen over

breast-feeding, recommendations are given regarding their compositions (section 1.2), based on the composition of human milk.

1.1.2. Human and bovine milk

The raw material most extensively used for decades for the production of infant formulae is bovine milk (Table 1.1; Blanchard et al. 2013). Consequently, a deep understanding of the composition of human milk is essential to most closely match the chemical composition and, increasingly, the nutrition and health outcomes of breast-fed babies (O’Mahony and Fox 2013; Crowley et al. 2016). At a macro level, the composition of human milk and infant formula can be considered as the macronutrients, proteins, lipids, carbohydrates and minor nutrients. The protein content and quality (i.e. digestibility and bioavailability) of infant formula is critical in influencing the growth and development of the infant (Blanchard et al. 2013). Protein in the formula will supply nitrogen and amino acids that are essential for the development of the infant. Bovine milk contains three times more protein than human milk, signifying that bovine milk-derived protein ingredients need to be recombined in ratios that will best match the protein content and profile of human milk, also considering the higher proportion of casein to whey (casein represents 80% of total protein for bovine milk compared to 40% for human milk), making raw bovine milk unsuitable for infants.

Macro ingredients (per 100 ml)	Human milk	Bovine milk
Total protein (g)	1.55	3.5
Casein (g)	0.85	2.8
Whey protein (g)	0.7	0.7
α -Lactalbumin (g)	0.35	0.2
β -Lactoglobulin (g)	0	0.35
Immunoglobulin (g)	0.15	0.05
Total fat (g)	3.5	3.6
Linoleic acid (% fat)	10	3
Cholesterol (mg)	20	13
Total carbohydrates (g)	7.5	4.5
Lactose (g)	6.5	4.5
Oligosaccharides (g)	1.0	Trace

Table 1.1. Comparison of the composition of human milk and bovine milk (data from Blanchard et al. (2013))

Beyond total protein content, another important aspect to consider is the quantity of each essential and semi-essential amino acid. In this regard, the FAO/WHO declare that, for an equal energy value, the formula must contain an available quantity of each essential and semi-essential amino acid at least equal to that contained in the reference protein (human milk, as defined in Table 1.2). Individual free amino acids may be added to infant formula, but only to improve its nutritional value for infants. Essential and semi-essential amino acids may be added to improve protein quality, but only in amounts necessary for that purpose (FAO 1981).

	% Amino acid in protein	
	Human milk	Bovine milk
Alanine	4.0	3.2
Arginine	4.0	3.4
Aspartic acid	8.3	7.2
Cystine	1.7	0.8
Glutamic acid	17.8	19.8
Glycine	2.6	1.9
Histidine	2.3	2.6
Isoleucine	5.8	5.8
Leucine	10.1	9.5
Lysine	6.2	7.6
Methionine	1.8	2.5
Phenylalanine	4.4	4.7
Proline	8.6	9.2
Serine	5.1	5.1
Threonine	4.6	4.6
Tryptophan	1.8	1.3
Tyrosine	4.7	4.8
Valine	6.0	6.2

Table 1.2. *Amino acid content of human and bovine milk protein*

Another major macronutrient to consider in the formulation of infant nutritional products is lipids, which provide 40–50% of the daily energy intake. The lipid fraction of infant formula is usually incorporated in the form of blends of different

vegetable oils in order to best match the fatty acid profile of human milk (Blanchard et al. 2013). Concerning carbohydrates, the main constituents of human milk and infant formula are oligosaccharides and lactose, and to best match human milk, significant fortification with lactose is needed in bovine milk-based formulae (for detailed information on the composition of infant formulae and the ingredients used, see section 1.3). It is also important to appreciate that while infant formulae are based on human milk composition, this knowledge base is continually developing and evolving, with new scientific information constantly becoming available (Hemmingway et al. 2020; Caldeo et al. 2021).

1.2. Types of infant formula and respective regulations

Foods for infants and young children collectively represent a wide range of products from birth to three years. These products may be divided into five major categories: first-age infant formula, second-age or follow-on formula, cereal-based and baby foods for infants and young children, young child formulae and formulae for special medical purposes. Each of these nutritional product categories is governed by different regulations, having different protein and amino acid requirements, among other important considerations such as carbohydrates, lipid content and classes, and concentrations of micronutrients such as minerals and vitamins (Table 1.3).

1.2.1. *Infant or first-age formula*

Infant milk, or first-age infant formula (0–6 months), is an industrially produced, highly formulated, nutritionally complete, human milk substitute, designed for infant consumption during the first months of life through to the introduction of appropriate complementary feeding (Koletzko et al. 2005; O’Callaghan et al. 2011). It is typically based on bovine or caprine milk and other ingredients (e.g. lipids, carbohydrates and minerals), which have been proven to be suitable for infant feeding. Infant formula attempts to mimic the nutritional profile of human breast milk and is the only food, other than human milk, that the medical community considers nutritionally acceptable when a mother cannot breast-feed or chooses not to breast-feed, or when the infant has some type of allergy/intolerance (Nasirpour et al. 2006; Thompkinson and Kharb 2007). Regarding the protein composition of infant formulae, bovine and caprine milk proteins (whey and caseins), soy proteins and their respective hydrolysates are permitted for the production of infant formulations (FAO 1981).

The protein content is typically between 13 and 15 g/l, and varies depending on the type and quality of the protein; for example, a minimum of 1.8 and a maximum of 2.5 g of protein per 100 kcal apply in the case of bovine and caprine milk-based formulae. On the contrary, infant formula manufactured from soy protein isolate alone, or as a mixture with bovine or caprine milk protein, or protein hydrolysates, must contain a higher minimum protein concentration of 2.25 g or 1.86 g per 100 kcal, respectively, and a maximum of 2.8 g per 100 kcal for both intact protein and hydrolysates.

For soy protein-based infant formulae, only protein isolates should be used, and the minimum protein content required by European legislation (European Commission 2016a) is higher than that of bovine milk protein due to the lower digestibility and consequently lower bioavailability of soy protein compared with intact bovine milk protein (Agostoni et al. 2006). The reason for having higher minimum protein levels when plant-derived proteins or protein hydrolysates are used is the lower amino acid quality of plant proteins, compared with dairy sources. Some plant proteins are deficient in certain indispensable amino acids, and the digestibility of plant proteins is generally lower than that of milk proteins. Therefore, a higher minimum protein content is usually recommended for formulae containing intact proteins other than milk proteins, whereby the amino acid composition of the infant formulae will be closer to that of the reference protein (i.e. breast milk) with increasing protein concentration.

By around 4–6 months of age, exclusive reliance on breast milk or infant formula no longer provides sufficient essential nutrients necessary for the infant's growth and development (Cichero 2016). Complementary feeding, as defined by the WHO, is “the process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants” so that “other foods and liquids are needed, along with breast milk” (WHO 2001). Therefore, formulae with different composition must be introduced to the infant to meet the increased demand for energy and nutrients (Table 1.4), which is imposed by rapid growth (Food Safety Authority Ireland 2011). The sequence of such introductions most often followed is iron-fortified infant cereal (rice), followed by vegetables, fruit and finally animal protein, such as meat. Because of a perception that the infant's relatively permeable intestinal tract would allow uptake of foreign proteins, provoking allergic reactions, it is customary to limit the allergenic load by using a single-grain cereal as the first food. Gluten-containing foods are often introduced after six months because they are more likely to cause allergic reactions (Lucas and Zlotkin 2003).

Age	Stage	Source of protein	Energy (kcal/100 mL)	Protein (g/100 kcal)	Carbohydrates (g/100 kcal)	Lipids (g/100 kcal)	Regulation
0–6 months >6 months	First-age infant formulae	Cow or goat Soya Protein hydrolysates	60–70	1.80–2.5	9.0–14.0	4.4–6.0	EU 2016/127
	Second-age or follow-on formulae		60–70	2.25–2.8 1.86–2.8	9.0–14.0 9.0–14.0	4.4–6.0 4.4–6.0	
3–6 years	Cereal-based and baby foods	Processed cereal-based foods - Simple cereals reconstituted with milk or other appropriate nutritious liquids - Cereals with added high-protein food reconstituted with water or other protein-free liquids - Pasta - Rusks and biscuits Baby foods - Meat, poultry, fish, offal – main ingredients - Meat, poultry, fish, offal – combination with other ingredients - Cheese - Desserts and puddings - Fruit juices	Each product has its own minimum and maximum values for protein, carbohydrate and lipid content according to the regulation 2006/125/EC				
1–3 years	Young child formulae	Young child formulae are governed by specific legislation					

Table 1.3. Types of infant nutritional formulations, sources of protein and macronutrient composition thereof

	Infants		Children	
			Toddlers	Young children
			Weaning	
	0–6 months	6–12 months	1–3 years	4–8 years
Total water (L/d)	0.7	0.8	1.3	1.7
Carbohydrate (g/d)	60	95	130	130
Total fiber (g/d)	–	–	19	25
Fat (g/d)	31	30	-	-
<i>Linoleic acid</i> (g/d)	4.4	4.6	7	10
<i>α-Linolenic acid</i> (g/d)	0.5	0.5	0.7	0.9
Protein (g/d)	9.1	11	13	19

Table 1.4. *Dietary reference intakes (DRIs) for infants (0–12 months) and children (1–8 years). Sources: Food and Nutrition Board (2011); Joint WHO/FAO/UNU Expert Consultation (2007)*

1.2.2. Follow-on or second-age formula

Follow-on, or second-age formula, is a food intended for use as part of the weaning diet for infants from 6–12 months and for young children (1–3 years), respectively, and can be prepared from bovines or other animal milk, and/or other constituents of animal and/or plant origin (FAO 1987; European Commission 2006b). Formulae developed for the second six months of life are designed as complementary foods for infants who have been introduced to some solid food, with the objective of providing a superior nutrient source compared with bovine milk (O’Callaghan et al. 2011). The protein content of such products should not be less than 1.8 and not more than 3.5 g per 100 kcal for bovine and caprine milk protein, and a minimum of 2.25 and a maximum of 3.5 g per 100 kcal for protein hydrolysates and soy protein isolate-based formulae. As in the case of infant formulae, the quantity of each essential and semi-essential amino acid must be at least equal to that contained in the reference protein, in this case breast milk (Table 1.2).

1.2.3. Cereal-based foods and baby foods

1.2.3.1. Cereal-based foods

Cereal-based foods are used as a complementary food for infants, generally from the age of six months onwards, as a supplement to their diet and/or for their progressive adaptation to ordinary food. These are prepared primarily from one or more milled cereals such as wheat, rice, barley, oats, rye, maize, millet, sorghum and buckwheat. They may also contain legumes, starchy roots or starchy stems or oil seeds in smaller proportions. This category is broad, ranging from simple products, such as cereals, that can be reconstituted with water or milk, to more complex formulated products, such as snacks, non-milk-based drinks, puddings, biscuits and pasta. The amount of cereal in such products should not be less than 25% of the final mixture, and the protein content should not exceed 5.5 g/100 kcal and should not be less than 2.0 g/100 kcal. The chemical index of the added protein should be equal to at least 80% of that of the reference protein, in this case the casein from bovine milk (Table 1.5); alternatively, the protein efficiency ratio of the protein in the mixture should be equal to at least 70% of that of casein (European Commission 2006a).

	g/100 g of protein
Arginine	3.7
Cystine	0.3
Histidine	2.9
Isoleucine	5.4
Leucine	9.5
Lysine	8.1
Methionine	2.8
Phenylalanine	5.2
Threonine	4.7
Tryptophan	1.6
Tyrosine	5.8
Valine	6.7

Table 1.5. *Amino acid composition of casein
(from European Commission (2006a))*

1.2.3.2. *Baby foods*

Baby foods, like cereal-based foods, are intended for infants and young children that are being weaned, as a supplement to their diet and/or adaptation to ordinary food. These food products are more similar to those intended for adults and represent a transition food. These can include meat, poultry, fish or other traditional sources of protein.

1.2.3.3. *Formulae for young children*

Formulae for young children (also referred to as growing-up milk, toddler milk or milk-based drinks for young children) are not specifically defined in EU legislation and are not included within the existing specific measures applying to foods intended for infants and young children. Until July 19, 2016, Directive 39/EC (European Commission 2009) provided regulatory guidance for young-child formulae. According to the new legal framework, young-child formulae fall within the scope of Directive 1925/EC (European Commission 2016d); in particular, they must meet regulatory requirements related to vitamins and minerals. Apart from this Directive, these products must also comply with other relevant EU regulations that apply to all types of foods, such as Regulation (EC) 178/2002 (general principles of food law and food safety) and Regulation (EC) 1924/2006 (nutrition and health claims made on foods).

Formulae intended for young children can be described as specifically processed/formulated protein-based drinks intended to satisfy the nutritional requirements of young children aged from 1–3 years. While the number of individual manufacturers is small, there are hundreds of different young-child formulae products available commercially on the EU market (European Commission 2016b).

The European Commission proposes not to use the term “growing-up milk” as this would imply a particular effect on growth and also proposes not to use the term “toddler milk” as it considers that a “young child” is better defined by age. Consequently, “young-child formula” is the term proposed by the EFSA Panel in 2016 for Formulae Intended for Young Children. This term also includes formulae based on protein sources other than bovine milk (e.g. soy-based growing-up milk). There is an increasing number of milk-based drinks and similar products on the EU market that are promoted as particularly suitable for young children, and the EU market for these products is growing.

Nutrient	Minimum (g/100 kcal)	Maximum (g/100 kcal)	Median (g/100 kcal)
Protein	2	6.7	2.6
Casein	0.1	2.4	1.7
Whey protein	0.4	1.2	0.7
Whey/casein ratio	0.5	6.3	1.5
Carbohydrates	7.3	15.4	12.6
Sugars	3.1	13.7	9.9
Lactose	0.1	13.5	9
Sucrose	0.6	10.4	2.1
Glucose	0	1.8	0.5
Maltose	0.1	5	0.2
Maltodextrin	1.4	11.2	4.1
Fat	3	5.7	4.3
Saturated fat	0.2	4.3	1.4

Table 1.6. *Average nutrient composition of young-child formulae (data from Perez et al. (2013))*

Such products can be formulated using protein of animal or vegetable origin such as bovine milk, goat milk, soy or rice. In most cases, bovine milk is used as a protein source, but the protein content of the product is normally lower than that of bovine milk (3.5%) (Table 1.6) and in most cases is within the range permitted by the legislation for infant formulae and follow-on formulae (European Commission 2016b; McCarthy et al. 2016). They are often fortified with several micronutrients (e.g. iron and vitamin D), polyunsaturated fatty acids (e.g. alpha-linolenic acid) and other substances (e.g. taurine) that are commonly present in infant formulae and follow-on formulae, and in many cases not present (or present at lower concentrations) in bovine milk. Young-child formulae may contain different sugars (e.g. lactose, sucrose, glucose, maltose), sometimes honey, and in certain cases flavorings (e.g. vanilla), and nutritional superiority to bovine milk is often used as a competitive marketing position. In a scientific report, the European Commission (2016b) concluded that young-child formulae are one of the ways to increase the intake of n-3 polyunsaturated fatty acids, iron and vitamin D in infants and young children (which have been identified by the EFSA as nutrients, along with iodine, at

risk of inadequacy for some infants and young children in the EU). Some young-child formulae may contain substances (e.g. sugars, flavors) at concentrations that are generally not recommended for young children (considering the contribution of sugar consumption to obesity, or the impact of sugars or flavors on the development of taste in young children).

1.2.4. Available formats of infant and follow-on formulae

Infant formulae are available on the market in three forms (Crowley et al. 2017):

- powder: the least expensive form that must be mixed with water before feeding;
- liquid concentrate: must be mixed with an equal amount of water;
- ready-to-feed: the most expensive form of infant formula that requires no further mixing.

These three forms of infant nutritional products are commercialized in a variety of packaging formats (Table 1.7) to satisfy specific practical and medical needs.

1.2.5. Formulae for special medical purposes

Infant formulae for special medical purposes is another category of infant nutritional products that are manufactured to meet, by themselves, the special nutritional requirements of infants with certain disorders, diseases or medical conditions (e.g. low birthweight, lactose intolerance, premature infants) during the first months of life up to the introduction of appropriate complementary feeding. These products are developed to meet the nutritional requirements of infants and are adapted to the dietary management of such infant's specific conditions. Examples of such formulae include low-birthweight (whey-dominant), high-caloric or nutrient-dense (whey-dominant) and extensively hydrolyzed protein formula (casein/whey protein hydrolysate and rice protein hydrolysate-based formulae).

Regarding the protein composition, it should follow the same requirements as for first-age infant formula, except when it is necessary to modify it to meet the specific nutritional requirements of the infant (FAO 1981; O'Callaghan et al. 2011).

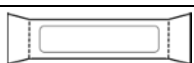
Format	Features of the format
 Powder in can	– Free-flowing bulk powder in multi-use can – Consumer scoops designated quantity for correct nutrient density – Dissolved in sterilized water/container prior to consumption – Reusable can
 Tablet	– Compressed powder in single-serve tablet – Correct nutrient density contained within tablet – Dissolved in sterilized water/container prior to consumption
 Stick pack	– Free-flowing powder in single-serve sachet – Correct nutrient density contained within sachet – Dissolved in sterilized water/container prior to consumption
 Pouch	– Free-flowing bulk powder in plastic pouch – Used to refill cleanable, reusable tubs/cans
 Plastic bottle	– Ready-to-feed/pre-sterilized liquid – Re-sealable container – Multi-use (within 48 h after opening)
 Glass bottle	– Ready-to-feed/pre-sterilized liquid – Heavier than other ready-to-feed formats – Single-use (within 48 h after opening)
 Tetra Brik	– Ready-to-feed/pre-sterilized liquid – Occupies less transit space than other ready-to-feed formats – Single-use (within 48 h after opening)
 Concentrate	– Concentrated liquid infant formula – Mixed with designated quantity of sterilized water to achieve correct nutrient density – Ease-of-preparation lies between the can format (intensive) and the ready-to-feed format (easy)

Table 1.7. Overview of the different formats of infant formulae commercially available (adapted from Crowley et al. (2017))

1.3. Ingredients

The first step involved in the manufacture of infant formula is the preparation of the bulk ingredients as a mixture in either water or liquid skimmed milk. All the major and minor nutrient contents are adjusted to closely match human milk composition (see section 1.1.2.), with some of the more heat-labile components (e.g. water-soluble vitamins) typically added later in the manufacturing process after heat treatment. In the case of bovine milk-based formula, the concentration and profile of the major proteins are typically adjusted by adding demineralized whey or whey protein concentrate to a skim milk base. Moreover, to best reflect the fatty acid profile of human milk, vegetable oil blends are generally used as the fat source, with the carbohydrate component consisting primarily of lactose, which is innately contributed by skim milk and whey ingredients. Soluble fiber ingredients and oligosaccharides are also added in certain formulations (Kelly and Fox 2016).

1.3.1. *Proteins*

The protein content and profile of human milk are very different from bovine milk, with human milk having considerably lower protein content and a higher proportion of whey protein than bovine milk (de Wit 1998). As a result, first-age infant nutritional products are specifically formulated using high-quality ingredients to achieve the target protein content and provide the required essential amino acids. These differences in protein content and profile have important implications for the kinetics of protein digestion and curd-forming properties under gastric conditions – human milk generally forms a softer and more flocculent curd, which contributes to easier digestion (Thompkinson and Kharb 2007) and also to differences in the kinetics of the release of amino acids and other nutrients (Crowley et al. 2017). The regulations state that the protein content of such products should not be less than 1.3 g/100 g and not more than 1.5 g/100 g. This value is higher than what is usually found in human milk (0.8–1.0 g/100 g) to compensate for the low levels of certain amino acids (tryptophan, tyrosine and cysteine) (European Commission 2006a). Thus, to meet the protein content and amino acid profile requirements, demineralized whey or whey protein concentrate are the most extensively used ingredients in first-age formulae (European Commission 2016b). In addition, several other types of whey-based ingredients are also suitable for infant formula applications, with the final selection being based on factors including nutritional, technological, marketing, compositional specification and price/functionality interrelationships (Fenelon et al. 2019). Conversely, follow-on formulas are generally formulated to be casein dominant as they are mostly used as substitutes for bovine milk (O’Callaghan and O’Mahony 2011).

In addition to meeting the compositional and regulatory requirements in formulating infant nutritional products, it is also important to consider that the protein profiles of human and bovine milk are considerably different. In fact, human milk does not contain β -lactoglobulin (β -lg) and has higher levels of α -lactalbumin (α -lac) and lactoferrin (Lf). In recent decades, the development of fractionation techniques has been a key driver in the development and commercialization of infant formulae whose protein profiles are more closely tailored to match those of human milk. In recent work, Fenelon et al. (2019) and Barone et al. (2020) reviewed the major whey proteins and their potential benefits in infant formula applications.

The amino acid sequences of α -lac in human and bovine milk have been shown to be 72% homologous, and with this high homology, α -lac derived from bovine milk is a suitable substitute for α -lac from breast milk in infant formulae. α -lac is rich in tryptophan and cysteine, hence using higher levels of α -lac allows formulators to meet amino acid requirements with reduced total protein levels. Davis et al. (2008) showed that infants fed formulae containing a lower level of total protein and a higher level of α -lac presented a closer gastrointestinal (GI) tolerance to that of breast-fed infants, with fewer GI events (constipation, gastroesophageal reflux, abdominal pain, vomiting, diarrhea and regurgitation) than infants fed with non-enriched formulae.

Likewise, human Lf has 77% homology with bovine Lf, suggesting that bovine Lf would be suitable for addition to infant formulae as it exhibits a number of important biological activities in infants (Goulding et al. 2021). For more information on bioactive proteins, the reader is referred to section 1.3.5.

β -Lactoglobulin is the major whey protein in bovine milk, but is not found in human milk. Apart from the provision of essential amino acids, the biological function of β -lg is unclear. Most commercial dairy-based infant formulae contain β -lg, where it importantly provides amino acids during digestion, while work such as that by Sanchon et al. (2018) has shown that β -lg is intact in human jejunal effluent after digestion, which has the potential to cause cow's milk allergy in some infants. Infants that are subject to intolerances and/or allergies, such as cow's milk intolerance/allergy (CMI/CMA) (El-Agamy 2007; Koletzko et al. 2012), require special medical formulations, with the protein platform of such formulae based on hydrolysates of whey and/or casein. Somewhat related to this are formulations designed for the treatment of IgE-mediated CMA, which are based on free amino acids. In addition, anti-regurgitation products containing starch or other thickeners, often being casein-dominant, are available to limit the incidence and severity of gastroesophageal reflux in infants.

1.3.2. Lipids

Lipids provide the main energy source in infant feeding, typically contributing 40–55% of the total energy (Koletzko et al. 2001). They are also involved in the regulation of a number of cell functions. Hence, the profile of lipids in infant formula directly influences gastrointestinal function, lipoprotein metabolism, membrane composition and function, thereby affecting infant growth, development and health (Mazzocchi et al. 2018). The fat profile of human milk is mainly composed of triacylglycerols (TAG), which consist of a glycerol backbone with three fatty acids (FA) attached at sn-1, sn-2 and sn-3 positions, with properties varying depending on the specific FA composition. Some of these FA are indispensable nutrients; polyunsaturated fatty acids (PUFAs) and long-chain PUFAs (LC-PUFA) such as docosahexaenoic acid (DHA) and arachidonic acid (ARA) must be supplemented to ensure appropriate development of the infant brain, retina acuity, electrophysiological response, improved immune status and problem-solving skills (Koletzko and Braun 1991; Makrides et al. 1994, 2009; EFSA Journal, 2009; O’Callaghan et al. 2011). Many studies have shown the importance of these LC-PUFA’s, as well as the importance of achieving a ratio of at least 2:1 ARA and DHA (Manley et al. 2011; Campoy et al. 2012; Sabel et al. 2012).

The fat component of most infant formulae is constituted by a mixture of vegetable oils (e.g. palm oil, coconut oil, sunflower oil). The TAG structure of vegetable oil and human milk fat differ by the distribution of palmitic acid at the sn-2 position, which is only 10–20% of all palmitic acid for vegetable oil and 70–88% in human milk fat (Sun et al. 2018). During digestion, hydrolysis of vegetable oil TAG releases free-FA from the sn-1 and sn-3 position, which combine with calcium, forming indigestible calcium soaps, and have been shown to result in reduced calcium absorption, energy loss, constipation and digestive discomfort (Mohan et al. 2020). Therefore, inter-esterification of vegetable oil TAG, which allows altered distribution of FA at the sn-2 position with the FA at the sn-1/sn-3 position, is implemented to produce TAG enriched in sn-2 palmitic acid (Hageman et al. 2019).

Dairy lipids were historically used in infant formula and are still used in some parts of the world, but their use has diminished (Delplanque et al. 2015). However, the incorporation of dairy fats along with vegetable oils in infant nutrition is receiving renewed attention, with recent work showing that adding dairy lipids presents an alternative approach for improving omega-3 FA status in infants (Giani et al. 2018). Lipids can be supplemented from different sources with the addition of single cell oils, fractionated lipids and various polar lipids, re-esterified structured lipids, egg phospholipids and fish oils (Delplanque et al. 2015).

1.3.3. Carbohydrates

The main carbohydrate (about 85% of total carbohydrate; Blanchard et al. 2013) present in human milk, and therefore in infant formulae, is lactose, which provides about 40% of total energy and has beneficial effects on infant gut physiology (Koletzko et al. 2005). However, other digestible carbohydrates are permitted by the FAO/WHO, such as maltodextrin, to improve the bulk handling and physico-chemical properties of the infant formula powders (Masum et al. 2019), or sucrose and glucose to enhance the organoleptic properties. Cooked starch can also be added for specific medical formula to confer increased satiety and less post-feeding regurgitation (Salvatore et al. 2017). In addition, other oligomers such as galacto-oligosaccharide (GOS) and/or fructo-oligosaccharide (FOS) can be added to infant formula. The non-digestible carbohydrates, GOS and FOS, are now commonly used in infant formulae for their prebiotic activity. They are not naturally present in human or bovine milk, but GOS is produced by enzymatic fermentation of lactose and sucrose, while FOS is extracted from chicory and both are added to infant formulae to modify the infant gut microbiota in order to be more similar to breast-fed infants by promoting the growth of *Bifidobacteria* (Bakker-Zierikzee et al. 2005).

1.3.4. Minor nutrients

Vitamins are present in human milk in the form of fat-soluble and water-soluble ingredients. Retinol (vitamin A), tocopherol (vitamin E), calciferol (vitamin D) and phyloquinone (vitamin K) are the fat-soluble vitamins, whereas the water-soluble vitamins are thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), cyanocobalamin (vitamin B12), ascorbic acid (vitamin C), folic acid (vitamin B9) and biotin (vitamin H or B8) (Montagne et al. 2009). The European Commission (2006) provides minimum and maximum requirements for each of these micronutrients to support infant growth and development. Indeed, a lack of vitamins would result in deficiencies for the infant, while a high intake of water-soluble vitamins has no negative impact as they are easily eliminated by the body. Conversely, an excess of fat-soluble vitamins has the potential to result in their accumulation in the tissues and may induce unwanted effects (Koletzko et al. 2005). Adequate mineral intake is essential to support infant development, as calcium, iron and zinc deficiency can result in sub-optimal skeletal mineralization, cognitive decrement and failure to thrive, respectively. Other minerals, such as phosphorus, magnesium, sodium, chloride and potassium, are nutritionally relevant for infant development and may be added to infant formulae as well as trace elements (manganese, iodine, selenium and copper). It should be

emphasized that the exact chemical form of each mineral can significantly influence their bioavailability. In addition, other ingredients (e.g. choline, taurine and nucleotides) may be added to ensure that the formulation is suitable as the sole source of nutrition for the infant or to provide other benefits that are similar to human milk (Joint WHO/FAO/UNU Expert Consultation 2007).

1.3.5. *Ingredient innovation for infant formula*

Research and innovation in ingredients for infant formula is greatly influenced by our evolving understanding of the chemical composition of the gold standard, human milk, and also by our desire to better match the physiological and health outcomes of human milk-fed infants. As a result, this will be dependent heavily on developments in analytical science and the application of novel analytical tools in the study of human milk to match its nutritional/functional properties.

1.3.5.1. *Innovation in dairy proteins*

Bioactive proteins, such as α -lac and If, are of considerable interest in infant formula, and it has been demonstrated that, beyond their basic nutritional contribution, they also contribute certain bioactivities such as mineral binding, anti-infective and immune modulation (Garcia-Montoya et al. 2012; Lyons et al. 2020). More specifically, it has been found that supplementation with α -lac promotes a plasma amino acid pattern similar to that of breast-fed infants (Kelleher et al. 2018), in addition to other similarities such as growth parameters, gastrointestinal tolerance and hormonal regulation (Lönnerdal 2014; Barone et al. 2020). In addition to these important physiological and health outcomes, α -lac-enriched formulas have greater heat stability and reduced protein–protein interactions (Davis et al. 2007; Crowley et al. 2016; Buggy et al. 2017), all of which are desirable in the formulation and processing of infant formulae. Concerning If, recent studies have focused on the impact of bovine If (blf) enriched infant formulae on iron bioavailability, infections and intestinal microbiota of infants. However, while If is essential to the development of a newborn's immune system, Grzywacz et al. (2020) showed that blf supplementation had minimal impact on the intestinal microbiota of infants, and Asztalos et al. (2020) showed that blf-supplemented formulas do not significantly reduce the mortality of low-birthweight infants. This limited impact of blf in infant formula could be due to its affinity with contaminants (e.g. lipopolysaccharide) present in commercial blf that could have blocked the bioactivity of blf (Lönnerdal 2014). Therefore, future studies on blf-enriched formulae should give particular attention to the detailed chemical composition of the commercial blf used. Heat treatment during processing of infant formulae has the potential to impact the bioactivity of lactoferrin (Goulding et al. 2021). Therefore, developing enhanced

understanding of the interrelationships between ingredient chemistry, processing and bioactivity is important (for more information on processing-mediated impacts on nutritional and functional properties, see section 1.4.).

β -Casein is another protein of interest in infant nutritional product formulation, and together with κ -casein, these two proteins are the most prevalent caseins in human milk. However, it is important to note that bovine β -casein is most frequently found in two variants, A1 β -casein and A2 β -casein, differing slightly by their amino acid profile; A2 β -casein being closer to that of human milk, which is of interest for humanization of infant formula. In fact, A1 β -casein may be associated with issues such as digestive disorders, immune disorders, type 1 diabetes and respiratory dysfunction, while A2 β -casein is claimed to be more similar to that in human milk and consequently reduces the risk of such disorders, and helps to maintain optimal growth and development (Sadler and Smith 2013).

1.3.5.2. *Fermentation-produced ingredients*

Human milk contains non-digestible oligosaccharides (HMOs), which have prebiotic effects and inhibit the binding of pathogens to cell surfaces, preventing the adhesion of a number of bacterial and viral pathogens, as well as protecting the gastrointestinal tract (O'Callaghan et al. 2011), and these HMOs are of considerable importance in infant health and development. 2'-Fucosyllactose (2-FL), one of the most abundant HMOs in human milk (nearly 30% of all HMOs; Vandenplas et al. 2018), may be produced by chemical synthesis, with microbial fermentation having become a viable and cost effective platform for production of 2-FL (Bych et al. 2019). *Escherichia coli* is commonly used as the host organism for fermentation; however, a recent study (Yu et al. 2018) suggested the use of *Saccharomyces cerevisiae* for safer production. Other HMOs, such as lacto-N-tetraose, are receiving interest due to their presence in a non-negligible quantity in human milk (Baumgärtner et al. 2015).

1.3.5.3. *Plant proteins*

The world faces a major challenge in food production and environmental sustainability over the next 30 years, coinciding with an expected growth of the global population to more than nine billion people by 2050 (Aiking 2011; Day 2013). Therefore, a significant increase in the demand for, and consumption of, food is anticipated. Being conscious of the environmental impact of such a demand (Heller et al. 2013), it is important to conduct research into new, innovative and more sustainable food products. It is widely recognized that diet choice is strongly related to environmental impact, and in this regard, plant-based diets are believed to have a lower environmental impact than animal-based diets (Springmann et al.

2016). In addition to these considerations, allergies and intolerances to bovine milk (Novembre and Vierucci 2001) are other factors that promote research activities to focus on plant-based products. The most common plant-based infant formulae on the market use soy proteins, as, according to the FAO/WHO, only soy protein should be used as a plant protein source for infant formula. However, other plant-based infant formulae, such as rice, are available commercially (Bocquet et al. 2019) and prototypes have also been developed using other plant protein sources, such as lentil (Alonso-Miravalles 2020). Even with the development and commercialization of soy protein-based infant nutritional products to support infants that cannot tolerate bovine milk-based products due to CMI/CMA, other intolerances or different considerations, it has been suggested that the consumption of soy-based products at an early stage in life could influence the hormonal development of the baby and contribute to certain disorders (Upton et al. 2019; Mvondo et al. 2019). Also, there is no evidence supporting the use of soy protein formulae for the treatment of infantile colic, regurgitation or prolonged crying (Agostoni et al. 2006). In line with health and environmental controversies, recent studies have questioned the use of soy in Europe (Rekow 2019; Rausch et al. 2019; Gollnow et al. 2018). Hence, these health and environmental considerations have led to innovation in infant formulae using new plant-based protein products classified into five sources (Norton et al. 1978; Alonso-Miravalles and O'Mahony 2018):

- cereals (e.g. barley, wheat, maize, rice, oats);
- legumes (e.g. chickpea, soya bean, pea, lentil);
- oil seeds (e.g. rapeseed, sunflower, cotton);
- roots and tubers (e.g. potato, yam, cassava, sweet potato);
- pseudocereals (e.g. amaranth, buckwheat, quinoa).

Notwithstanding the environmental and sustainability criteria, there are other important considerations for using these ingredients in infant formula. Indeed, vegetable-based proteins are all of lower protein quality than dairy, meat and fish-based proteins due to their fiber content, the presence of anti-nutritional factors and their different protein molecular structures (Moughan 2017). Seed proteins have lower overall nutritional quality than animal proteins, which can be related to their low content of sulfur-containing amino acids, the compact proteolysis-resistant structure of the native seed proteins and the presence of anti-nutritional compounds, which may affect the digestibility of proteins themselves and other components (Duranti 2006). Anti-nutrients have been defined as substances that by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Francis et al. 2001). Anti-nutritional factors may occur naturally or may be formed during heat

processing of protein products, yielding Maillard compounds, oxidized forms of sulfur-containing amino acids, D-amino acids and lysinoalanine (an amino acid derivative) (Gilani et al. 2012). There are many anti-nutritional compounds that can affect protein and mineral utilization, such as trypsin inhibitors and hemagglutinins in legumes, tannins in legumes and cereals and phytates in cereals and oilseeds (Francis et al. 2001; Alting and Van De Velde 2012). Those that affect protein utilization and digestion are mainly lectins, protease inhibitors, tannins and phytates (Gilani et al. 2012). Anti-nutritional factors are present in certain protein sources (e.g. soybean meal, peas and fava beans) and have been reported to increase losses of endogenous proteins at the terminal ileum (Salgado et al. 2002).

However, the interest in plant-protein is growing, and recent research has focused on new and emerging proteins for use in the formulation of infant nutritional products. Recently, potato protein has been investigated for its application in infant nutritional formulations. This hypo-allergenic protein has very good emulsifying properties and heat stability, particularly on modification of its properties (e.g. microparticulation) (Nestec 2019). Other alternative protein sources being investigated are legumes (e.g. pea, lentil, fava bean and chickpea), oil seeds (e.g. sunflower and canola) and pseudocereals (e.g. quinoa, amaranth and buckwheat), which can provide nutritional and functional properties similar to soy. Indeed, recent scientific studies by Le Roux et al. (2020a, 2020b) investigated the production of infant formula with a combination of dairy and plant proteins (i.e. rice, fava bean, potato and pea). Infant formula made with potato protein showed high viscosity, while rice protein had low solubility and was difficult to process. The work showed that pea and fava bean proteins are particularly promising for the production of infant formula in terms of their technological properties and in vitro digestibility.

1.4. Technological options for manufacture of infant nutritional products

1.4.1. *Powders versus liquids*

Infant nutritional products can be classified into two physical forms, powders and liquids, with each format prepared using different processing options, sharing some unit operations.

1.4.1.1. *Powdered infant formula*

Powdered infant formula can be produced using two core manufacturing approaches, “dry-blending” which involves the mixing of dry ingredients without heat treatment, and “wet-mixing” (Figure 1.1) (Montagne et al. 2009), which

involves the recombination of water-soluble ingredients in milk or water to meet the desired macro chemical composition, followed by pre-heating and addition of oils and emulsifiers (if used). A stabilized emulsion is normally obtained by homogenization and heat treated to inactivate all potential pathogenic organisms, followed by evaporation and spray drying (Westergaard 2004; Pisecký 2012).

Choosing one approach over the other is influenced by multiple considerations; however, in general, it is important to consider that the dry processing approach (although much less widely practiced) requires less energy and involves lower capital expenditure, while wet processing generally involves higher capital and operating costs. Furthermore, through the dry processing approach, the microbiological and physical quality depends on the raw material and integrity of the actual dry blending process, and, without agglomeration, bulk-handling properties of the blended product are determined largely by the properties of individual ingredient powders, with the possibility of segregation of ingredients occurring during transportation.

Even though they can be used separately and present specific advantages or disadvantages, the two approaches are often combined, as minor ingredients, such as dried vitamins and minerals, can be added directly after spray drying for dry blending. In such a case, special attention is given to the microbiological quality and safety of those ingredients. In any case, exact formulations, as well as specific technological approaches used for production of infant formula, are commercially sensitive and vary considerably between companies.

1.4.1.1.1. Recombination

Recombination occurs in two different ways, where water and oil-soluble ingredients are hydrated separately before combining them. Water-soluble powders are added to milk or water to achieve rehydration in several stages: wetting, sinking, dispersion and dissolution (McSweeney and Fox 2009), very often supported by using an in-line high shear mixing device to aid in the complete hydration of powder constituents (O'Sullivan et al. 2017), as well as increasing the mixing temperature (i.e. $\leq 50^{\circ}\text{C}$) (Bylund 1995; Guo 2014). Minerals are then added to the water-soluble ingredients mix and the pH of the solution can be adjusted to ensure its stability to subsequent heat treatment ($\sim\text{pH } 6.8$) (Montagne et al. 2009). Finally, oil-soluble ingredients are dispersed in the oils and blended with the wet mix. Water-soluble vitamins can be added as a cold premix to the homogeneous mix before spray drying or as a dry premix to the dried powder.

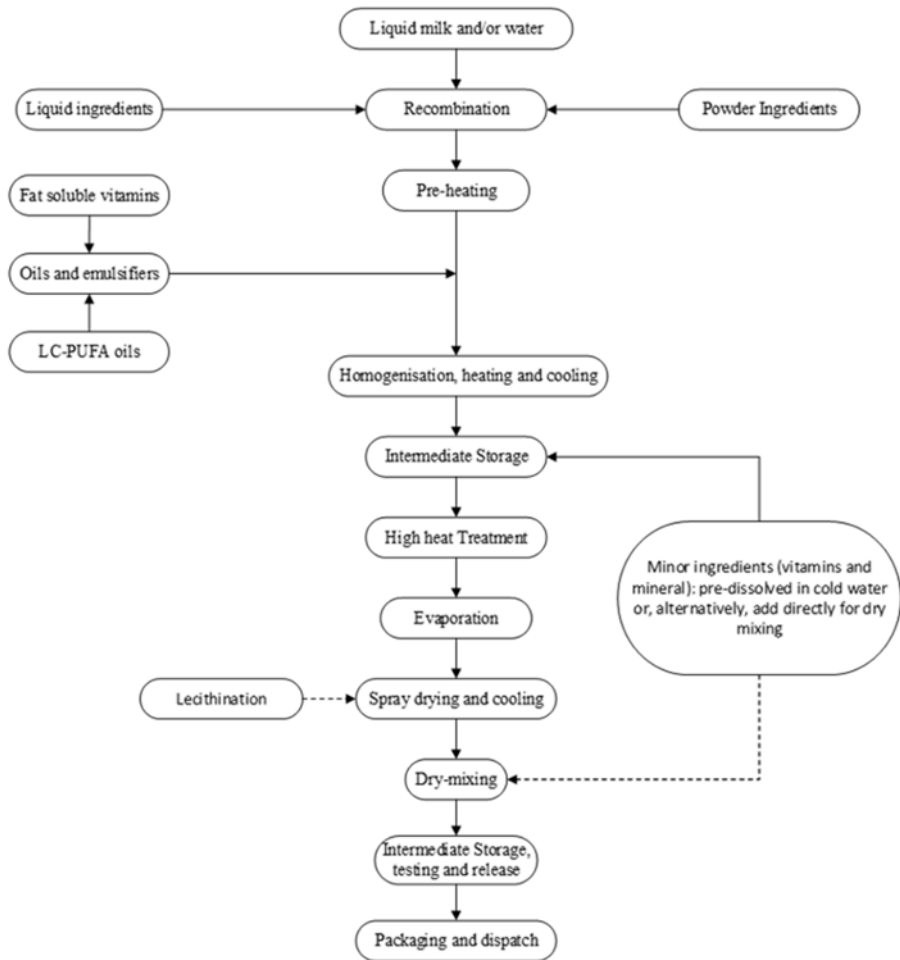


Figure 1.1. Schematic representation of the manufacture of dried infant formulae. The dotted lines represent alternative processing routes (Montagne et al. 2009)

1.4.1.1.2. Homogenization

A stable emulsion is essential to ensure adequate functional properties of the powder after spray drying. To achieve this, homogenization is normally performed using two-stage valve homogenizer technology, applying first and second stage pressures of ~14 and ~3 MPa, respectively (McCarthy et al. 2012; Murphy et al. 2015; Masum et al. 2019). A higher pressure at the first stage would lead to smaller fat globules and possibly result in instability and cavitation. The pressure at the

second stage ensures the disruption of any fat clusters formed during the first stage. In order to obtain an emulsion as stable as possible, it has been suggested that the droplet size after homogenization should be less than 1 μm (McSweeney 2008). The proteins present in the wet mix are amphiphilic and are the principal surfactants in most infant nutritional products. Nevertheless, non-protein emulsifiers (e.g. lecithin) are often added to help proteins/peptides form and stabilize emulsions (McSweeney et al. 2008; Singh 2011). The ratio of whey to casein proteins in the wet mix has been shown to influence emulsification, with previous work showing that a higher proportion of casein (60:40) results in a higher concentration of protein adsorbed at the interface compared to an emulsion made with whey protein isolate (WPI) alone (100:0), with important implications for emulsion stability (Sourdet et al. 2002; McCarthy et al. 2012). Furthermore, Dalglish et al. (2002) demonstrated that casein is preferentially adsorbed over whey proteins at the oil–water interface and Buggy et al. (2017) reported that increasing the proportion of α -lac in IMF emulsions retards the aggregation of β -lg, conferring greater stability on resultant emulsions.

1.4.1.1.3. Heat treatment

Thermal processing may be performed on liquid infant formula mixes before or after homogenization to ensure microbial quality in the finished product, as well as to achieve targeted physiochemical properties (McSweeney et al. 2004, 2008; McCarthy et al. 2012, 2013). In terms of sequence of unit operations, Buggy et al. (2017) suggested that heating before homogenization would be preferred, allowing for physical disruption of any aggregated fat globules/fat–protein complexes created during heating. In thermally treating infant formula mixes, two principal formats of heat treatments are used: direct heating, which consists of direct contact between the heating medium and the product (steam injection or infusion), and indirect heating, which involves using a (tubular or plate) heat exchanger. Direct treatment is preferred due to the rapidity of treatment as well as the limited denaturation of β -lg and α -Lac or to avoid the evaporation stage, with the added advantage of not having a physical heat exchange surface to limit fouling, while indirect treatment is preferred due to lower energy consumption. On the contrary, direct treatment may cause cavitation-induced damage and requires more maintenance, while indirect treatment can result in solution instability and a more intense Maillard reaction (Datta et al. 2002; McSweeney et al. 2004; Tran et al. 2008; Lewis and Deeth 2009).

When using these technologies, the protein profile of the product needs to be considered, as for example, whey proteins are heat-labile ingredients (Fenelon et al. 2019), hence their physico-chemical properties are impacted by heat-induced denaturation (Mulcahy et al. 2017). In addition, as they are the dominant protein in IMF ($\approx 60\%$ of total proteins), the denaturation of whey proteins will impact the thermal sensitivity of the wet mix. β -lg is heat sensitive due to the presence of a free

sulfhydryl group that unfolds on heating and interacts with itself, as well as other whey proteins and casein micelles, resulting in the formation of protein aggregates (Fenelon et al. 2019). However, it is important to note that hydrophobic interactions also contribute to the creation of aggregates (Manderson et al. 1998). α -Lac is more stable to thermal processing than β -lg; indeed, the former protein does not contain free thiol groups. However, self-aggregation within α -lac monomers has been shown to be reversible (McGuffey et al. 2007). Caseins, which represent 40% of the total protein in infant formulae, are significantly more heat stable than whey proteins, but have the ability to interact with whey proteins (Guyomarc'h et al. 2009; Kehoe and Foegeding 2014; Murphy et al. 2015; Singh et al. 2019).

1.4.1.1.4. Evaporation

Evaporation is used to achieve a more cost-effective removal of water than spray drying, as well as to increase the total solids before spray drying, which directly influences the physical properties of the resultant powders (Fox et al. 2010). It is a process in which the homogenized wet mix flows by gravity through a falling film evaporator and forms a thin film inside of each tube that composed it, in which steam is applied on the outer surface of the tubes, causing the evaporation of water from the wet mix (Morison et al. 2006). The process is performed under vacuum in order to keep the wet mix at a relatively low temperature (50–70°C). The liquid viscosity is the limiting factor during evaporation, as the concentrated mix needs to be fed to the spray dryer afterwards; indeed, it is generally recommended that the viscosity of the wet mix after evaporation should be in the range of 60–100 mPa.s (Westergaard 2004).

1.4.1.1.5. Spray drying

After evaporation, the concentrated mixture is dehydrated by spray drying to produce an infant formula powder as it is using efficient and gentle drying technology (O'Callaghan and Wallingford 2002). Multi-stage spray dryers are generally used to enable the production of a high-quality powder with good bulk-handling properties such as agglomeration, solubility, dispersibility and wettability (Blanchard et al. 2013). The stabilized mixture is fed on top of the spray dryer into a nozzle or wheel atomizer that atomizes it into fine droplets (10–400 μ m) in the main chamber (180–200°C) to increase the contact surface with the hot air (Westergaard 2004; Montagne et al. 2009). At this stage, most of the water is removed, and the formed particles fall by gravity into an internal fluidized bed (50–60°C) at the bottom of the main chamber of the spray dryer where additional drying takes place. An additional external fluidized bed (20–30°C) can be added to complement or replace the internal one (where relevant), with such external fluidized beds also facilitating cooling of the produced powder and their agglomeration (Hazlett et al.

2021). Full control of the parameters during spray drying is essential because the rapid evaporation of water leads to the migration of milk components to or from the surface of the drying droplet, thus influencing the structure of the powder particles and their rehydration properties (Kim et al. 2003). Therefore, spray drying, as described above, may change and will require specific optimization for each product due to the variety and complexity of the ingredients involved.

1.4.1.1.6. Infant formula powder composition

The high temperatures used in spray drying have little effect on the denaturation of whey proteins (Guyomarc'h et al. 2000; Haque et al. 2015), as the particles only ever reach a temperature of approximately 70°C due to the evaporative cooling effect, thus limiting the denaturation of whey proteins. However, casein micelles are highly hydrated structures and drying changes the micelle structure, resulting in higher hydrophobicity and possible insolubility (Baldwin 2010). Concerning fat, it has been widely shown (Fäldt and Bergenståhl 1994; Kim et al. 2009; Foerster et al. 2016) that when dairy powder contains a high level of fat (such as infant formula powders), the particle surface is mainly composed of fat, resulting in poor wettability of the powder and immiscibility of the particles with water. The most commonly applied solution to this problem is instantanization with lecithin (Hammes et al. 2015), known as lecithination. Lactose is highly impacted by the spray-drying process, with lactose forming an amorphous glass that reduces molecular mobility and restricts the movement of the encapsulated fat (Baldwin et al. 2010; Zhou and Roos 2011). However, certain conditions (reaching the glass transition temperature, T_g) during spray drying could lead to a transition to a rubbery state of lactose, leading to greater mobility of molecules and a higher level of surface free fat. A high concentration of lactose in its rubbery state could also cause stickiness and caking, causing damage to the spray-dryer as well as decreasing powder quality (Chuy and Labuza 1994; Vuataz 2002; Roos 2002; Nasipour et al. 2006). Reaching the glass transition of lactose will also increase the crystallization of lactose during storage and decrease powder flowing ability (Fitzpatrick et al. 2004).

1.4.1.2. *Liquid infant formula*

Liquid infant formulae are available in two states: ready-to-feed that can be used as it is and concentrates that require mixing with water. These products represent a small minority of the infant formula market as they are relatively expensive formats and are available mainly for convenience reasons or to support specific medical conditions, such as preterm or low-birthweight infant formula (Happe and

Gambelli 2015), as the manufacturing of liquid infant formula (Figure 1.2) results in a commercially sterile product. The early steps, from recombination to homogenization, are similar to the production of powdered infant formula with the following few differences. The recombination can be achieved in a batch or continuous mixing system, often under vacuum, to avoid possible foaming that can occur and induce issues with subsequent steps. Homogenization is achieved as in the production of infant formula powder and is normally accompanied by additional flash cooling. The resulting stabilized mixture is then standardized, sterilized and undergoes intermediate aseptic storage, allowing more control of the flow, as well as traceability before the final aseptic storing (Montagne et al. 2009).



Figure 1.2. Schematic representation of the manufacture of liquid infant formulae (Montagne et al. 2009)

1.4.2. Innovation in processing technologies for infant formula

Several innovations in infant formula have come through to market in the areas of ingredient and formulation innovation, and it is true that in recent years, important innovations have been realized through the adoption of new processing technology and powder treatments. Some research areas are focused on new automated options for infant formula reconstitution, such as the use of a collaborative robot (CoBoT) to better understand the human biomechanical movements used when preparing an infant's bottle (O'Shea et al. 2021) or providing new physical product formats such as tablets, which combine some of the benefits of powder and liquid product formats in a single physical format (Ndife et al. 2004). Another example of an interesting innovation was the development of the BabyNes[®] beverage dispensing apparatus by Nestlé, which provides parents with a method of preparing infant formula using single-use capsules, allowing for a more convenient and time-effective preparation (Kutcher and Kutcher 2016). Another approach is to investigate the inter-relationships between processing parameters and ingredients on the processing stability of formulae. For example, Drapala et al. (2016) studied the effect of protein hydrolysate–maltodextrin conjugates as emulsifiers in model infant formula emulsions, and showed that thermal and storage stability could be enhanced. Other recent studies (Barone et al. 2019; France et al. 2020; Wang et al. 2020; Arzuaga et al. 2021; Saxena et al. 2021) have shown success in addressing the high-level challenge of improving the processing stability and functional properties of infant formula through formulation.

The development of more energy-efficient and sustainable processing practices has been increasingly investigated over the last decade. Furthermore, due to the relatively low heat stability of a number of bioactive and nutritional ingredients, interest in low heat load processes has increased. High-pressure processing (HPP), ultraviolet irradiation, ultrasound and microwave treatments are all promising technologies that present an alternative to conventional thermal treatment (O'Sullivan 2015; Aceti et al. 2020; Harouna et al. 2020; Zhang et al. 2020). Other technologies such as pulsed electric field (PEF) processing and ohmic heating (OH) aim to ensure the microbial stability of the formulae with electrical energy to inactivate pathogens and enzymes. The latter have shown promising results as they allow better stability of thermosensitive ingredients, as well as better organoleptic properties due to their low-heat treatment (PEF) or rapid and uniform heating (OH) (Vanga et al. 2018; Pires et al. 2020). Reducing energy consumption and costs can also be achieved through process innovation to reduce the number of steps necessary for the production of infant formula. For example, certain lipids and fat-soluble heat-labile vitamins are added immediately before drying using in-line injection technology to reduce homogenization costs, while preserving the nutritional and

functional properties of the ingredients. In an effort to address some of these challenges, Murphy et al. (2011) developed a direct heat-treatment shock wave technology using high velocity steam injection, which allows for increased colloidal stability of high solids emulsions and lower whey protein denaturation (Murphy et al. 2013), suitable for spray drying, while bypassing the evaporation step by recombination at high total solids. Another important unit processing operation used in the production of infant formula is microfiltration. More specifically, microfiltration has demonstrated the ability to enhance the microbiological quality of raw materials for use in infant formulae (e.g. reduction of microbiological spore counts in skim milk powder), as well as for use in the chemical-free, sustainable fractionation of whey proteins from caseins in skim milk (McCarthy et al. 2017; France et al. 2021) for use in the formulation of next-generation infant nutritional products.

1.5. Conclusion

For over a century, the primary objective in formulating infant nutritional products has been to match the composition of human milk as much as possible, and in recent years has shifted towards achieving a better match of the nutrition and health outcomes of human milk-fed infants. This progressive humanization of infant formula continues to be influenced by our evolving knowledge of human milk, growth and development of infants and processing efficiency, sustainability and environmental considerations. In response to these considerations, innovations in ingredients (e.g. plant proteins), processing optimizations (e.g. enhancing heat stability), the development of new processing technologies (e.g. shock wave technology) and indeed the development of new product formats (e.g. tablets) will play a role in providing very interesting research and commercial activities in the coming years.

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