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# 1 Milk fat components and milk quality

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## 1.1 INTRODUCTION

From a physico-chemical point of view, milk is an emulsion of lipid globules and a colloidal suspension of protein and mineral aggregates in a solution of carbohydrates (mainly lactose). In Western countries, milk and dairy products, and in general food of animal origin, are often accused of causing adverse health effects, especially with regard their food lipid intake, since lipids have been implicated in several diseases such as obesity, insulin resistance and atherosclerosis (Olofsson et al., 2009). For these reasons, the number of studies on the physical and chemical structure of fat in several edible products of animal origin have increased. Although milk and dairy products contain saturated fatty acids, they also provide specific beneficial components for human health and also lipid components (phospholipids, some individual fatty acids (FAs) and fat-soluble vitamins) that have a role in health maintenance. In addition, milk is a major source of dietary energy, especially in developing countries, where there is shortage of animal-source food (FAO, 2013), and in childhood.

Milks of different origins have long been used, and they have been processed to dairy products for their longer shelf life. Due to the wide natural variability from species to species in the proportion of milk macronutrients and to variations along lactation, milk represents a flexible source of nutrients that may be exploited to produce a variety of dairy products.

Ruminant milk is the main source available for humans to use to manufacture dairy products and fermented milk. Besides cow's milk and milk from other ruminants (such as buffalo, goat and sheep), research on milk from other species is still poorly exploited (FAO, 2013). More recently, equine milks have been suggested for use in children with severe IgE-mediated cow milk protein allergy (CMPA) (Monti et al., 2007, 2012; Sarti et al., 2016), and local producers have established a niche for the application of donkey products with well-characterised profile of its constituents (Martini et al., 2014a).

### 1.1.1 Milk fat globules

Milk lipids are composed of milk fat globules (MFGs) made up of triglycerides enveloped by a biological membrane. MFGs are responsible and/or contribute to some properties and phenomena in milk and dairy products and may affect milk fatty acid composition and the way in which fat is digested (Baars et al., 2016; Huppertz and Kelly, 2006; Martini et al., 2017). For the dairy industry it is of interest that changes in the morphometry of the MFGs lead to changes in milk quality, yields, and ripening and the nutritional quality of cheeses (Martini et al., 2004).

In milk of different species there are MFGs of various sizes, ranging from a diameter smaller than 0.2  $\mu\text{m}$  to a maximum of about 15  $\mu\text{m}$ , with an average diameter that varies as a function of endogenous (species, breed), physiological (parity, stage of lactation), and exogenous factors (feeding) (Martini et al., 2010a).

Different average diameters have been reported in the literature for ruminant species (3.5–5.5  $\mu\text{m}$  for cows; 2.79–4.95  $\mu\text{m}$  for sheep; 2.2 and 2.5–2.8  $\mu\text{m}$  for goats and 2.96–5.0  $\mu\text{m}$  for buffalos) (Table 1.1) (Martini et al., 2016b). However average diameter of globules in equids is considerably lower than other dairy species (about 2  $\mu\text{m}$  in donkey) (Martini et al., 2014b), while regarding human MFGs, larger dimensions have also been found (4  $\mu\text{m}$ ) (Lopez and Ménard, 2011).

The MFG membrane (MFGM) is a triple membrane resulting from the mammary secretory cell that surrounds a core of triglycerides distributed in a lamellar way (Heid and Keenan, 2005).

The MFGM consists of different classes of lipids (phospholipids, triglycerides and cholesterol) and of several proteins and enzymes. Phospholipids, in the form of mixtures of fatty acid esters of glycerol and sphingosine, possibly containing phosphoric acid, and a nitrogen-based compound (choline, ethanolamine or serine). These are natural emulsifiers able to maintain the milk lipids as discrete globules, ensuring high stability. MFGM contains about 1% of the total milk proteins. Most of them are present in very low amounts and are enzymes and proteins involved in milk synthesis. The principal proteins in the MFGM include mucins (MUC) 1 and 5, adipophilin (ADPH), butyrophilin (BTN), periodic acid-Schiff glycoproteins (PAS) 6 and 7, fatty acid binding protein (FABP), and xanthine oxidoreductase (XOR), a metal (Mo, Fe) binding protein (Spertino et al., 2012). In the last few years, research on the composition and structure of the milk membranes have been increased and have improved the knowledge of the MFGM from species other than the bovine (Saadaoui et al., 2013; Pisanu et al., 2012; Lu et al., 2016; Martini et al., 2013).

These studies have increased also due to the fact that MFGM is a dietary source of functional substances and is considered a nutraceutical (Rosqvist et al., 2014; Timby et al., 2015; Hernell et al., 2016). The functionality of the MFGM seems to be provided by its content of phospholipids, sphingolipids, fatty acids and proteins with an antibacterial effect (such as xanthine oxidoreductase and mucins) and/or health benefits.

MFGM conveys fat in an aqueous environment and is damaged by some treatment, such as homogenization, whipping and freezing, affecting milk physicochemical properties, for example producing hydrolytic activity, rancidity, and oiling off, and low wettability of milk powders. MFGM composition also affects the creaming rate on the milk surface (Martini et al., 2017); in bovine milk this phenomenon is due to the effect

**Table 1.1** Average values in literature for fat content, milk fat globules characteristics and fatty acid composition of milk from different species.

		Cow	Buffalo	Goat	Sheep	Donkey	Horse	Human
Fat	%	3.70	8.14	3.90	6.50	0.36	1.48	3.34
Average diameter of the fat globules	µm	3.5–5.5	2.96–5.0	2.2–2.8	2.79–4.95	2	2–3	3.3
SFA	g/100g fat	71.24	65.9	70.42	71.85	55.55	45.18	41.77
MUFA	g/100g fat	25.56	31.4	25.67	26.04	22.21	31.88	38.73
PUFA	g/100g fat	3.20	2.70	4.08	2.10	21.08	22.93	16.96
UFA	g/100g fat	28.76	34.1	29.75	28.14	43.29	54.81	55.29
UFA:SFA ratio		0.40	0.52	0.42	0.39	0.78	1.20	1.32
SCFA	g/100g fat	10.52	9.72	17.51	17.13	12.29	10.79	1.87
MCFA	g/100g fat	52.81	53.70	48.28	45.87	40.08	42.47	37.94
LCFA	g/100g fat	34.38	32.73	32.64	35.87	47.64	46.75	57.72
CLA c9, t11	g/100g fat	0.65	0.45	0.70	1.00	–	0.09	0.19
C18:2 n6 (LA)	g/100g fat	2.42	1.71	2.72	1.20	9.5	16.17	12.96
C18:3 n3 (ALA)	g/100g fat	0.25	0.51	0.53	0.77	7.25	5.96	1.15
C18:2 n6: C18:3 n3 ratio	–	9.68	3.35	5.13	1.56	1.31	2.71	11.26
C20:4 (AA)	g/100g fat	0.13	0.10	0.16	0.10	0.09	0.10	0.4
C20:5 (EPA)	g/100g fat	0.05	0.03	nd	nd	0.26	–	0.11
C22:6 (DHA)	g/100g fat	nd	–	0.05	0.04	0.28	–	0.51

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; SCFA: short chain fatty acids ( $\leq 10\text{C}$ ); MCFA: medium chain fatty acids ( $\leq 11\text{C}$ ,  $\geq 17\text{C}$ ); LCFA: long chain fatty acids ( $\geq 18\text{C}$ ); nd: no data.

of cryoglobulins, an M-type immunoglobulin that aggregates globules during cold storage. Other types of milk are lacking these cryoglobulins and do not agglutinate. Homogenization reduces globule diameter, making globules insensitive to the action of cryoglobulins and prevents agglutination. During butter production, extensive agitation and kneading causes the MFGM to form the water-in-oil emulsion. The partitioning in the aqueous phase produces the loss of MFGM in the buttermilk.

### 1.1.2 Milk fat and fatty acid composition

Milk lipid content and fatty acid composition vary by virtue of various endogenous and exogenous factors. Among endogenous factors, the species, breed and stage of lactation are the main factors.

Regarding the species, buffalo and sheep milk contains higher fat percentages and are particularly suitable for processing, such as cheese making. Fat percentages vary in a range between 7 and 9% for buffalo, but can reach 15% under favourable conditions (Altomonte et al., 2013; Varricchio et al., 2007), whereas in sheep the range is between 6.5 and 9% depending on the breed (Haenlein, 2007; Martini et al., 2012). Regarding cow and goat milk, fat content are comparable; in fact cow total lipid ranges from 3.4% in Holstein to about 6% in Jersey breeds (Nantapo et al., 2014; Pegolo et al., 2016; Sanz Ceballos et al., 2009), and goat range from a minimum of 3.5% to a maximum of 5.6% in some native goats (Haenlein, 2007; Martini et al., 2010b). Equid milk has lower fat percentages compared to ruminant milk; the average values reported in literature are 0.30–0.53% in donkey and 1.5% in horse milk (Pikul and Wójtowski, 2008; Martemucci and D'Alessandro, 2012; Martini et al., 2014b; Salimei et al., 2004). Furthermore, some authors stated lower contents (1.04%, 0.92%, 0.8%) in the milk of Halfinger, Hucul and Wielkopolski mares, respectively (Salamon et al., 2009; Pieszka Huszczyński and Szeptalin, 2011). The low fat content in equid milk could be a limiting factor in its use in infant nutrition in a diet exclusively based on milk, thus an appropriate lipid integration should be introduced. On the other hand it is encouraging for studies on the possible use of donkey milk in dietotherapy.

Regarding human milk, fat content is more similar to cow milk, varying between 2.8 and 3.8% (Antonakou et al., 2013).

From a nutritional point of view, donkey milk leads to lower saturated fatty acid (SFA) intake, about 2.00 g/l (Table 1.2), than the other milks commonly used for human feeding. Despite being rich in unsaturated fatty acids (UFAs) and having a UFA:SFA ratio intermediate between ruminant and human milk, donkey provides a limited amount of fat; thus, the total intake of UFA per 1 l of milk is lower (1.56 g) than milk of other species (Martini et al., 2016a).

In milk from ruminants, especially sheep and goats, triglycerides contain short chain fatty acids (SCFAs) such as butyric acid and hexanoic, octanoic and decanoic acid. On the contrary, human (Yuhás, Pramuk and Lien, 2006) and donkey milk (Martini et al., 2014b) are characterized by low amounts of SCFA—especially the chains shorter than C8—and high quantities of long chain fatty acids (LCFAs).

SCFAs are synthesized by the fermentation of dietary fibre, are water soluble and volatile, and contribute to the typical flavour of ovine and caprine milk. When freed by endogenous lipase or bacterial enzymes, SCFA can also give rancidity and quality

**Table 1.2** Calculated average values for fat content and some fatty acids (g/l) in milk from different species and Dietary Reference Values.

		Dietary Reference Values							
		Cow	Buffalo	Goat	Sheep	Donkey	Horse	Human	
Fat	g/l	Adults: 20–30% of the energy of the diet (E); Infants (6–12 months): % 40 E%; Children (2–3 years): 35–40 E%.							
SFA	g/l	26.36	53.64	27.46	46.70	2.00	6.68	13.95	
MUFA	g/l	9.45	25.56	10.01	16.93	0.80	4.71	12.94	
PUFA	g/l	1.18	2.20	1.59	1.36	0.76	3.39	5.66	
UFA	g/l	10.63	27.76	11.60	18.29	1.56	8.1	18.60	
C18:2 n6 (LA)	g/l	0.89	1.39	1.06	0.78	0.34	2.39	4.33	
C18:3 n3 (ALA)	g/l	0.09	0.41	0.21	0.50	0.26	0.88	0.38	
C20:4 (AA)	g/l	0.05	0.08	0.06	0.06	0.006	0.01	0.03	
C20:5 (EPA)	g/l	0.02	0.02	–	nd	0.009	–	0.04	
C22:6 (DHA)	g/l	–	–	0.02	0.03	0.010	–	0.17	
				(20% of AI)	(30% of AI)	(9% of AI)		(170.34% of AI)	
C20:5+C22:6	g/l	–	–	–	–	0.017	–	0.21	
(EPA + DHA)						(6.80% AI)		(84% of AI)	

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; nd: no data.  
Source: Data from EFSA (2010).

deterioration. SCFAs and MCFAs, which are a source of rapidly available energy, are particularly relevant for people suffering from malnutrition or fat absorption syndromes and for elderly people (Raynal-Ljutovac et al., 2008).

Recent studies have highlighted effects of SCFA at cellular and molecular levels in the organism; their presence or their deficiency may affect pathogenesis of some diseases (autoimmune, inflammatory diseases). In addition, SCFAs have antimicrobial activity and anti-inflammatory effects in the gut (Tan et al., 2014).

Ruminant milk, in particular milk from sheep that feed in pastures, is the richest natural source of conjugated linoleic acids (CLA) and of C18:1 trans-11 (vaccenic acid) (Bauman and Lock, 2005; Lim et al., 2014). The CLA content in milk varies depending on species, breed and individual, farming system, feeding and season. In sheep milk CLA varies from 1.2 to 2.9 g/100 g of fat; in goat between 0.5 and 1 g/100 g of fat (Parodi, 2003). Cow milk is generally reported to vary from 0.1 to 2.2 g/100 g total FA (Elgersma, Tamminga and Ellen, 2006), whereas human and equid milk are poor sources of CLA (Table 1.1).

Ninety percent of CLA isomers in milk is made up of *cis*-9, *trans*-11 C18:2 (rumenic acid) produced mainly by stearoyl Co-A desaturase (SCD) *o*- $\Delta$ 9-desaturase enzyme in the mammary gland using vaccenic acid as precursor, but also by the rumen bacterium *Butyrivibrio fibrisolvens* as intermediate of biohydrogenation of linoleic and linolenic acids ingested with feed (Bauman and Lock, 2005). Rumenic acid vary between 0.29 and 0.71% of total human milk fatty acids, while in the horse it is between 0.07 and 0.10%. Moreover, in equids, cecum seems to contribute little to CLA synthesis (Markiewicz-Keszycka et al., 2014).

Anticarcinogenic properties and modulation of immunological functions have been demonstrated for rumenic acid in animal models and cell cultures (Field and Schley, 2004; O'Shea et al., 2004). However, the most documented effects of CLA in humans are the gain of muscle mass at the expense of body fat, whereas *in vivo* studies on the effects on atherosclerosis and cholesterol have shown conflicting results in humans (Crumb, 2011).

Vaccenic acid has shown anticancer properties in human mammary adenocarcinoma cells (Lim et al., 2014).

Regarding the omega-3 FAs, milk is not a good source of this family of FAs. However, among the mammalian species reared for milk production, horse, sheep and donkey are richest sources of C18:3 n3 ( $\alpha$ -linolenic acid (ALA))(g/l) (Table 1.2), in particular donkey and horse milk provide a good ALA intake (0.22–0.88 g/l) although they have low fat content. In adults minimum intake levels for ALA are recommended to prevent deficiency symptoms (0.5% of energy) (FAO-WHO, 2010).

Linoleic acid (LA) and ALA are precursors of omega 6 and omega-3 families, respectively, and their ratio is generally considered as indicative of their balanced intake in the diet. The interest in the LA:ALA ratio derives from the antagonistic effects between the two families of FAs observed in human body. In fact, the higher intake of *n*-6 fatty acids may reduce the formation of anti-inflammatory mediators from omega-3 fatty acids. Observations on animal models suggest that raising the *n*-6 to *n*-3 fatty acids ratio (*n*6:*n*3) acts on adipogenesis and the risk of obesity in the offspring later in life (Rudolph et al., 2015). However, research is yet not supported by studies in humans, and an optimal ratio of these fatty acids in the diet has not yet been established (EFSA, 2010).

Furthermore, the prevalence of *n*-6 in human diets has increased over the decades while *n*-3 fatty acids remain unchanged, thus increasing the *n*-6/*n*-3 milk fatty acid ratio (Rudolph et al., 2015). Thus, a reduction of omega-6 in the diet is desirable, and donkey's milk appears to have a balanced rapport of these two families (about 1) compared to other milks (Martini et al., 2014b).

Arachidonic acid (AA) C20:4 is essential component of cellular membranes and also of MFGM, where it may have an essential role (Fong et al., 2007; Martini et al., 2013).

AA is present in almost similar amounts in the milk of ruminants (Table 1.2), while it shows lower values in equids.

Despite the importance of AA for membrane integrity (Fong, Norris and MacGibbon, et al., 2007), it has been described as an adipogenetic-, pro-inflammatory- and hypertension-promoting factor (Vannice and Rasmussen, 2014), and recommended intake levels have not been established.

C20:5 (EPA) and C22:6 (DHA) have showed evidence of both independent and shared effects in neuroprotection and in the treatment for a variety of neurodegenerative and neurological disorders. In particular, DHA is an important constituent of the retina and the nervous system, and it has unique and indispensable roles in neuronal membranes (Dyall, 2015).

There is still insufficient evidence to support beneficial effects of EPA and DHA in foetal life or early childhood on obesity, blood pressure, or blood lipids (Voortman et al., 2015).

Overall levels of DHA and EPA in milk are quite low, and in human milk DHA content is highly variable; values from 0.17 to 0.99 % have been reported, depending on the diets and on different countries (Yuhás, Pramuk and Lien, 2006). The recommended daily intake of EPA plus DHA is 0.25 g in adults (EFSA, 2010).

## 1.2 CONCLUSIONS

The transformation of milks of different origin may be the source of dairy products with different and peculiar characteristics. Since a role in health maintenance has been reported for several lipid components of milk, a deep knowledge of milk lipid constituents from different dairy species is of utmost relevance for both the nutritional uptake and effects on human health.

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