Microbiology in Dairy Processing

Challenges and Opportunities

Edited by

Palmiro Poltronieri

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Microbiology in Dairy Processing

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Foreword

Microbiology in Dairy Processing: Challenges and Opportunities is directed to the following: dairy scientists; dairy professionals in industry and academia; those in food science, dairy science and microbiology; intermediate course and post-graduate students; trained laboratory personnel; and R&D and production personnel in dairy industry companies of all sizes. The idea to write this book came from the section "Questions" in the Researchgate community. I realised that there is a need to introduce lactic acid bacteria (LAB) growth media at various levels of expertise, from young researchers starting their laboratory work to food technologists devoted to microbiological analyses. Therefore, from this starting point, I searched the recent literature and produced a list of exceptionally interesting publications on how far the genomics field has advanced in its knowledge of LAB species in recent years. The chapters in this book reflect these advancements and offer a panoramic view of the research fields in which to apply these advancements in knowledge, either for LAB and dairy-associated species and their applications in dairy productions and for the technologies to maintain the milk products safe and devoid of undesired pathogens and milk spoilage bacteria. The challenges of dairy microbiology are either to maintain the product safety devoid of undesired bacteria that may spoil the quality and change the taste or to the further advancement in the microbiota and the interaction among bacteria at community level. The opportunities remain in the exploration of the biodiversity of LAB and dairy-associated species, either at genome rearrangements and horizontal gene transfer or at the biochemistry level, to produce novel dairy products that are low fat, low salt, or with beneficial properties for human health.

Preface

Microbiology in Dairy Processing: Challenges and Opportunities introduces and reviews the knowledge regarding dairy technologies and lactic acid bacteria (LAB) and dairy-associated species in the fermentation of dairy products for laboratory technicians and researchers and students learning the protocols for LAB isolation and characterisation. It provides application notes useful in laboratories of food technology departments and for students and researchers studying all aspects of the milk-processing industry, from microbiology to food productions.

The chapters deal with the industrial processing of milk – the problems solved and those still affecting the processes, from microfiltration to deterioration of stored milk in cold by psychrotrophic bacteria (such as *Pseudomonas fragi*) and by spore-forming bacteria – and cheese-manufacturing technologies. The book introduces culture methods and species-selective growth media to grow, separate and characterise LAB and dairy-associated species, molecular methods for species identification and strain characterization, Next Generation Sequencing for genome characterization, comparative genomics, phenotyping, and current applications in dairy and non-dairy productions, as well as the potential future exploitation of the culture of novel strains with useful traits (probiotics, fermentation of sugars, metabolites produced, bacteriocins).

Chapter 1 introduces the quality and properties of milk fats and differences in milks of various origin. Chapter 2 overviews the spore-forming bacteria associated with milk. Chapter 3 discusses the problem of psychrotrophic bacteria in milk deterioration. Chapter 4 presents the various types of industrial milk according to the freshness and quality. Chapter 5 presents the advancements in LAB and dairy-associated species genomics and strain differences, related to gene content and their applications. Chapter 6 presents very broadly the biochemistry of LAB and dairy-associated species. Chapter 7 reviews selective growth media for different species of LAB and non-LAB dairy-associated bacteria. Chapter 8 introduces the molecular tools for strain identification and characterization. Chapter 9 discusses the bacteriocin-producing LAB species and their potential applications in food products. Chapter 10 analyses in detail the complex interactions among starter and non-starter strains. Chapter 11 reviews the physical-chemical properties of milk cream products and technological processes involving milk fats and cream-derived products. Chapter 12 analyses technological traits of lactic acid bacteria, their industrial relevance and new perspectives. Chapter 13 overviews LAB bacteriophages in dairy products, their problems and solutions. Chapter 14 details the application of LAB as a cell factory for delivering functional biomolecules in dairy products. Chapter 15 reviews the dairy technologies applied to yogurt production. Finally, Chapter 16 introduces properties of milk proteins, the differences in amino acids of protein variants, and the potential to originate bioactive peptides and the proteolysis

by co-fermenting LAB species, a process that may ensure the safety and healthiness of the fermented products, as assessed by EFSA authority. Last, the potential for milks of different origin to be administered to individuals suffering of milk allergies or intolerance is discussed.

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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).

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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 μm to a maximum of about 15 μm, 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 μm for cows; 2.79–4.95 μm for sheep; 2.2 and 2.5–2.8 μm for goats and 2.96–5.0 μm 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 μm in donkey) (Martini et al., 2014b), while regarding human MFGs, larger dimensions have also been found (4 μm) (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 (≤10C); MCFA: medium chain fatty acids (≤11C, ≥17C); LCFA: long chain fatty acids (≥18C); 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 (Yuhas, 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%.	37.0	81.4	39.0	65.0	3.60	14.8	33.4
SFA	g/l	as low as possible	26.36	53.64	27.46	46.70	2.00	6.68	13.95
MUFA	g/l	Not set	9.45	25.56	10.01	16.93	0.80	4.71	12.94
PUFA	g/l	Not set	1.18	2.20	1.59	1.36	0.76	3.39	5.66
UFA	g/l	Not set	10.63	27.76	11.60	18.29	1.56	8.1	18.60
C18:2 n6 (LA)	g/l	Adequate Intake (AI): 4 E%	0.89	1.39	1.06	0.78	0.34	2.39	4.33
C18:3 n3 (ALA)	g/l	AI: 0.5% E	0.09	0.41	0.21	0.50	0.26	0.88	0.38
C20:4 (AA)	g/l	Not set	0.05	0.08	0.06	0.06	0.006	0.01	0.03
C20:5 (EPA)	g/l	Not set	0.02	0.02	–	nd	0.009	–	0.04
C22:6 (DHA)	g/l	Infants and young children (between 6 and 24 months) AI: 0.10 g DHA	–	–	0.02 (20% of AI)	0.03 (30% of AI)	0.010 (9% of AI)	–	0.17 (170.34% of AI)
C20:5+C22:6 (EPA+DHA)	g/l	Adults. AI: 0.25 g	–	–	–	–	0.017 (6.80% AI)	–	0.21 (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*-11C18:2 (rumenic acid) produced mainly by stearoyl Co-A desaturase (SCD) *o*-Δ9–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 (α-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 (Yuhas, 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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