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15
Sensory and Flavor Characteristics of Milk

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15.1 INTRODUCTION

Milk from ruminant species is one of the main components of the human diet, especially in Western countries, because of its balanced nutritional composition and its versatility to be transformed into a great diversity of products. Although cows' milk is the main contribution to global milk production, in some countries, such as India and Pakistan, the majority of milk is produced by buffaloes, whereas in the Mediterranean region the production of milk by small ruminants such as sheep and goats is of considerable importance compared with that of cows' milk (Manfredini & Massari, 1989; Fox, 2011). Distinct differences in physicochemical properties have been reported among milks from ruminant species, and between milks from ruminant and monogastric species (Doreau & Martin-Rosset, 2002; Park & Haenlein, 2006). These compositional differences lead inevitably to distinctive sensory characteristics. Milk as discussed here refers to cows' milk unless otherwise stated.

Among sensory characteristics of milk, flavor is one of the most important attributes for acceptability and preference by consumers (Thomas, 1981; Kim & Morr, 1996). It is also a key attribute for obtaining quality products since it is the common and basic ingredient for many formulated dairy and non-dairy foods (Drake *et al*., 2003; Lloyd *et al*., 2009a). Typical milk flavor is the result of a delicate balance of a wide number of compounds, some of them present at very low concentrations (Nursten, 1997). Milk flavor depends on the metabolism of the animal and interactions between the animal and its environment (Toso *et al*., 2002), and thus variations in this attribute are expected (Thomas, 1981). These changes can lead to different defects known as off-flavors, which are associated with an unbalanced volatile profile.

It is well known that the smell of raw milk is typical for each ruminant species, which is considered dependent on some quantitative differences in the volatile profile and on the presence of specific compounds associated with each type of milk (Moio *et al*., 1993a; Toso *et al*., 2002). Cow milk flavor has been extensively studied but research on flavor of small ruminant milk is scarce (Carunchia Whetstine & Drake, 2006).

From very early times, the dairy industry and research in dairy science and technology have made enormous efforts to obtain safe dairy products and to extend their shelf-life and improve quality, including quality consistency. Nowadays, these efforts have doubled as new products have to be developed to satisfy the growing demand of consumers, who require both safe and healthy products and appealing sensory properties (Chapman *et al*., 2001).

Thermal treatments are extensively applied to raw milk to reach different objectives: (i) to ensure food safety by destroying pathogenic microorganisms, and (ii) to stabilize the product by inactivating enzymes and killing spoilage microorganisms, which prolongs shelf-life and allows the products to be stocked at room temperature (de Wit & Nieuwenhuijse, 2008; Hougaard *et al*., 2011). Pasteurization,

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ultrapasteurization (UP), ultra-high-temperature (UHT) process, in-container sterilization, and spray drying are the most extended thermal treatments in the dairy industry. The quality of thermally treated milks is greatly determined by the onset of unpleasant flavors which occur when processing variables deviate from appropriate conditions or when more stringent conditions of temperature/time are applied. The incidence of objectionable cooked flavor in pasteurized milk decreased considerably with the adoption of continuous processing methods such as high-temperature short-time pasteurization (HTST) (Thomas, 1981). UHT sterilization can promote strong sulfurous, cooked, cabbage-like flavors in milk, thus limiting its acceptance (Vazquez-Landaverde *et al*., 2006a). A precise control of processing conditions can reduce the appearance of this defect. Sensory changes in processed milk can also occur as a consequence of other factors including raw milk quality, post-processing contamination, storage conditions, and the use of certain packaging material that accelerate enzymatic and chemical reactions (Celestino *et al*., 1997a, b; Simon & Hansen, 2001a; Solano-Lopez *et al*., 2005; Smet *et al*., 2008). Specific volatile compounds have been linked to typical defects that develop in heated milks through storage, and the physical agents or chemical and enzymatic reactions that cause them have been established. For example, flavors of UHT milk described as cooked and cabbagey appear during the first stage of storage, and their intensity is dependent on the presence of volatile sulfides liberated via heat denaturation of the protein β-lactoglobulin (Simon & Hansen, 2001a; de Wit & Nieuwenhuijse, 2008). Short-chain free fatty acids released by lipases from milk fat can cause rancid flavors (González-Córdova & Vallejo-Cordoba, 2001). Stale flavors can be produced by several compounds originating mainly from non-enzymatic browning in milk powder (Karagül-Yüceer *et al*., 2002) or from lipid oxidation such as methyl ketones, linear-chain aldehydes, and sulfur compounds in UHT milk (Jeon *et al*., 1978; Rerkrai *et al*., 1987).

In recent years, non-thermal technologies have been explored to meet new demands of consumers about fresher and more natural products. They are required to ensure food safety with a minimum change in sensory characteristics. Technologies such as microfiltration, ultrasonication, pulsed electric field, microwave, and high pressure, alone or in combination with minimal thermal treatments, have been applied to milk. The effect of these promising technologies on volatile profile and flavor is still being investigated, although the preliminary results are encouraging (Trujillo *et al*., 2002; Clare *et al*., 2005; Vazquez-Landaverde *et al*., 2006b; Riener *et al*., 2009; Zhang *et al*., 2011).

Over recent decades large amounts of information have been accumulated on flavor and volatile compounds of milk from different species. Factors that can cause variations in the volatile profile and sensory characteristics of milk, from its biosynthesis to its sale, have been investigated. Most attention has been focused on off-flavor due to the economic impact that it implies. Other topics, such as the mechanisms and metabolic pathways that explain the origin of volatile compounds, or the impact of new milk-processing technologies are more recent research approaches or have not been elucidated.

15.2 SIGNIFICANCE OF FLAVOR AND OFF-FLAVOR ON MILK QUALITY: SENSORY AND INSTRUMENTAL METHODS

Milk flavor is one of the most important attributes for acceptability and preference by consumers (Thomas, 1981), being also a key attribute to obtain quality products (Drake *et al*., 2003; Lloyd *et al*., 2009a). Off-flavors have always been a major control problem for the dairy industry (Thomas, 1981), decreasing the sensory quality and economic value of products (Karagül-Yüceer *et al*., 2002). In addition, they can be a contributing factor in the decline of per capita consumption of milk (Leong *et al*., 1992).

Flavor is a complex sensation in which aroma plays the most important role, being mainly perceived by the interactions of the volatile components with the receptors of the olfactory epithelium in the nasal cavity (Nursten, 1997). Flavor cannot be measured directly by instrumental methods because it is the result of an interaction between food and consumer. Thus, milk quality evaluation has been commonly carried out by human assessment. Much research has attempted to develop objective tests for quality analysis (Horimoto & Nakai, 1998), since sensory methods are subject to errors from differences among individual assessments. Only the selection of an appropriate test and its correct application as well as accurate data interpretation leads to reproducible and relevant results (Drake, 2007). Excellent reviews on different aspects of sensory analysis of dairy foods, such as description of methods, when and how to use them and the information that can be obtained in each case, are available (Drake, 2004, 2007). Among sensory methods, descriptive sensory analysis is extensively employed in dairy products evaluation to identify and measure those attributes that best characterize their sensory properties. Its application requires both defined terms for sensory attributes and descriptors and trained panelists or judges, who can assess a certain product quality and detect changes due to off-flavors (Biolatto *et al*., 2007). Possible terms with references and accepted definitions for attributes and descriptors can be achieved through the development of a language (Drake & Civille, 2003). Descriptive sensory evaluation and flavor lexicons for fluid milk (Watson & McEwan, 1995; Chapman *et al*., 2001), milk powders

(Drake *et al*., 2003), dairy ingredients (Drake *et al*., 2003), and cheese (Muir *et al*., 1995; Drake *et al*., 2005; Liggett *et al*., 2008) have been proposed. A widespread lexicon addressed to off-flavor of milk was reported by the Committee on Off-Flavor Nomenclature and Reference Standards of the American Dairy Science Association (Shipe *et al*., 1978). This committee proposed seven categories of off-flavors (Table 15.1). In the USA and other Western countries these terms have been used extensively in formulating 100-point score cards for the evaluation of milk or cheeses and other dairy products in annual state and national judging competitions and products contests for high school [4-H youth clubs, FFA (Future Farmer of America) associations], collegiate and adult contestants, and product exhibitors (Nelson & Trout, 1964; Haenlein, 2000). The awarding of prizes and championship trophies for best-quality entries is inspiring for product quality improvement and very popular.

Objective tests for milk quality evaluation are not necessarily associated with consumer acceptance or preference. For this reason, consumer tests are widely used to determine both acceptability and shelf-life of a product (Drake, 2007).

Milk flavor is due to a complex mixture of volatile components in a specific matrix, consequently the analysis of the volatile fraction by instrumental methods becomes another important criterion in quality evaluation of dairy products (Nursten, 1997; Povolo *et al*., 2007), since it allows to identify objectively the presence and intensities of compounds linked to food flavor (Horimoto & Nakai,1998). Headspace techniques (static and dynamic headspace), solid-phase microextraction, distillation and/or solvent extraction (solvent-assisted flavor evaporation, simultaneous distillation extraction, etc.) are widely applied to characterize the aromatic fraction of milk and to evaluate the impact of different factors on the volatile profile.

The study of volatile fractions of milk by instrumental analytical methods is complex for several reasons: the majority of analytes are present at very low concentrations (Imhof & Bosset, 1994; Bendall, 2001) and the heterogeneous nature of the matrix of milk makes isolation difficult (Friedrich & Acree, 1998; Havemose *et al*., 2007). As many components are highly sensitive to heat, temperature extraction is critical to avoid generation of artifacts and to preserve the original flavor. In addition, it is not an easy task to establish the role of a single compound on overall flavor or to relate volatile compounds with desirable or undesirable sensory attributes. Finally, the concentration of a compound is not necessarily a measure of its sensory impact (Drake & Civille, 2003).

The onset of techniques combining olfactometry (O) and gas chromatography (GC) has enabled, at least partially, to overcome these difficulties (Friedrich & Acree, 1998). Their application to fresh and thermally treated milk samples has allowed identifying those components with a strong impact on flavor and to relate them with the characteristic flavor of each type of milk (Table 15.2). Electronic noses have been more adequate to distinguish normal samples from those with off-flavor (Marsili, 1999) or to monitor rancidity during storage (Capone *et al*., 2001).

Statistical tools are essential to find a correlation between sensory attributes and individual volatiles; they range from simple correlations to multivariate analyses such as factor analysis (FA), cluster analysis (CA), principal components analysis (PCA), and principal component similarity analysis (PCS) (Horimoto & Nakai, 1998).

15.3 MILK FROM RUMINANT SPECIES

15.3.1 Volatile profile and sensory characteristics of fresh milk

Sensory characteristics of milk from ruminants (cows, ewes, goats, etc.) differ from one species to another. Fresh cows' milk of overall good quality has a bland and clean but distinctive flavor, which has been described as a slightly salty-sweet taste and delicate aroma (Thomas, 1981; Bendall, 2001). The salty-sweet taste has been mainly attributed to milk salts and lactose but in fact, the aroma makes the most important contribution to flavor (Marsili, 2011). In addition, the sensory perception of bovine milk is significantly impacted by the pleasant mouth feel and aftertaste, which is due to the emulsion of milk fat (Badings & Neeter, 1980; Francis *et al*., 2005). However, since bovine milk possesses this bland and soft flavor, any deviation in this typical characteristic is readily perceived by consumers (Thomas, 1981; Shiratsuchi *et al*., 1994a). In contrast, the milk of small ruminants such as sheep and goats tends to have a more intense aroma characterized by waxy and animal notes. These attributes are not present in bovine milk. Milk fat composition plays a key role by defining its flavor (Carunchia Whetstine & Drake, 2006).

Both raw and pasteurized milk are usually considered "fresh milk" since the conventional pasteurization process (HTST) does not significantly alter the flavor or volatile compound profile (Moio *et al*., 1994; Nursten, 1997; Bendall, 2001; de Wit & Nieuwenhuijse, 2008). Consequently, in this chapter we will refer to raw and HTST pasteurized milk as "fresh milk." For few pasteurization processes, however, differences have been reported between raw and pasteurized milk: analysis of sheep milk by a sensory panel revealed that HTST pasteurized milk and untreated milk were not significantly different, but batch-pasteurized milk was described as muttony (Young, 1986). Another example: instant infusion pasteurization is often considered as a gentle process, but

Table 15.1. Categories of off-flavors proposed by Shipe *et al.* (1978).

Categories	Definition	Descriptive terms	References
Heated	Flavors that result from changes in milk components produced by thermal treatments	Cooked, heated, caramelized, scorched	Shipe *et al*. (1978)
Oxidized	Flavors produced from a reaction between molecular oxygen and polyunsaturated fatty acids, which can be induced by certain metals or light. In addition, this process may also occur spontaneously. Milk from some cows develops this defect so quickly, which is known as "spontaneous oxidation"	Oxidized, cardboardy, metallic, tallowy, oily, fishy	Shipe *et al*. (1978) Barrefors *et al*. (1995) Timmons *et al*. (2001)
Light-induced	Defects produced by light action on certain milk components. It has two distinct causes: one a burnt or sunlight flavor attributed to photodegradation of proteins and amino acids, which develops rapidly during the first days of storage and therefore become noticeable early, and a second component defined as metallic or oily that does not dissipate and becomes dominating in milk, similar to oxidized flavor, and attributed to lipid oxidation. In this case, photo-oxidation occurs in milk in the presence of a photosensitizer such as riboflavin, which excites oxygen to its singlet state by producing free radicals that can react with unsaturated fatty acids	Light, sunlight, activated, burnt, scorched, cabbage, old vegetable oil, cardboard, goat, metallic	Shipe *et al*. (1978) Jenq *et al*. (1988) van Aardt *et al*. (2001) Chapman *et al*. (2002) Moyssiadi *et al*. (2004) Havemose *et al*. (2004)
Lipolyzed	Flavors produced by hydrolytic release of free fatty acids from triglycerides by lipolytic enzymes. The particular action of native milk lipase (lipoprotein lipase) on cold stored milk fat is a process known as "spontaneous lipolysis"	Rancid, butyric, goaty, soapy	Shipe *et al*. (1978) Cartier & Chilliard (1990)
Microbial	Abnormal flavors that result from spoilage microorganisms or enzymes, which cause protein and fat degradation of milk	Acid, malty, fruity, putrid, unclean, sour, bitter	Shipe *et al*. (1978)
Transmitted	Flavors that may arise by passage of substances from the cow's feed to the mammary glands. This transfer may be via the respiratory and/or digestive system and bloodstream. In this category are also considered those flavors related to the migration into the milk of degradation products of plastic or residual solvent employed in packaging materials	Feed, weed, cowy, barny Unpleasant plastic	Shipe *et al*. (1978) Marsili (2011) Leong *et al*. (1992)
Miscellaneous	Flavors that either cannot be attributed to a specific cause or specifically defined in sensory terms	Absorbed, astringent, bitter, chalky, chemical, foreign, lacks freshness, salty	Shipe *et al*. (1978)

Source: based on data from Shipe *et al*. (1978).

Table 15.2. Main odor-active compounds reported in different types of milks.

Compounds	Odor descriptors*	Type of milk	References
Ethyl butanoate	Fruity, sweet, banana, fragrant	Bovine raw milk Ovine raw milk Caprine raw milk Buffalo raw milk	Moio *et al*. (1993a, 1994, 1996)
Ethyl hexanoate	Fruity, pineapple, apple, unripe fruit	Bovine raw milk Ovine raw milk Caprine raw milk	Moio *et al*. (1993a, 1994, 1996)
Heptanal	Green, sweet, herbaceous	Ovine raw milk Buffalo raw milk	Moio *et al*. (1993a, 1996)
Indole	Fecal, putrid, musty, floral in high dilution	Buffalo raw milk Caprine raw milk Ovine raw milk	Moio *et al*. (1993a, 1996)
Nonanal	Sweet, floral, green, grass-like	Buffalo raw milk Ovine raw milk Pasteurized bovine milk	Moio *et al*. (1993a, 1994, 1996)
1-Octen-3-ol	Mushroom-like	Buffalo raw milk Ovine raw milk Pasteurized bovine milk	Moio *et al*. (1993a, 1996)
Dimethylsulfone	Sulfurous, hot milk, burnt	Bovine raw milk Ovine raw milk Buffalo raw milk Pasteurized bovine milk UHT bovine milk	Moio *et al*. (1993a, 1994)
Hexanal	Freshly cut grass, green	Pasteurized bovine milk	Moio *et al*. (1994)
2-Heptanone	Blue cheese, spicy	UHT bovine milk	Moio *et al*. (1994)
2-Nonanone	Mustard like, spicy	UHT bovine milk	Moio *et al*. (1994)
2-Undecanone	Vegetable, floral, rose-like	UHT bovine milk	Moio *et al*. (1994)
Benzothiazole	Burning smell, rubbery	UHT bovine milk	Moio *et al*. (1994)
2-Tridecanone + δ-decalactone	Peach-like, floral	UHT bovine milk	Moio *et al*. (1994)

*Based on data from Moio *et al*. (1993a, 1994) and Friedrich & Acree (1998).

sensory properties of bovine milk subjected to this treatment were described by negative attributes such as cardboard, sour, and plastic, and its volatile fraction composition differed from HTST pasteurized milk (Hougaard *et al*., 2011).

Compounds involved in fresh milk flavor do not derive from a single source in the food. They can come from direct transfer from the cow's fodder, by absorption from the digestive tract (i.e. rumen and/or intestine) into the blood and thence to peripheral tissues such as the mammary gland. A second possible absorption is via the pulmonary route. In this case, compounds present in the air are inhaled by the ruminant or gases from the rumen absorbed in the lungs into the blood and from there they diffuse to the mammary gland. Finally, flavor compounds can be formed by metabolism (endogenous synthesis) in the rumen and/or by metabolic processes in the liver or mammary gland from carbohydrates, amino acids, fatty acids, and other compounds present in the fodder (Honkanen *et al*., 1964; Urbach, 1990a; Moio *et al*., 1996). In addition to those components naturally present in raw milk, a wide number of compounds can be produced by chemical or enzymatic reactions before and during dairy processing or induced during storage (Calvo & de la Hoz, 1992).

The most extensive research work available on volatile compounds of fresh milk was performed by Moio *et al*. (1993a, b, 1994, 1996), who analyzed milks from different ruminant species: cow, sheep, goat, and buffalo. In these studies, more than 80 volatile compounds belonging to different chemical classes, such as alcohols, ketones, esters, acids, sulfur, and nitrogen compounds, and aliphatic and aromatic hydrocarbons were detected. Volatile compounds identified were similar for the four

ruminant species, so results suggested that typical flavor for each ruminant milk mostly relied on quantitative differences. Regardless of this, certain compounds are considered as characteristic of each ruminant milk.

Among carbonyl compounds, methyl ketones, diketones, straight-chain aldehydes, branched-chain aldehydes, and aromatic aldehydes are frequently found in milk (Imhof & Bosset, 1994; Bendall, 2001; Toso *et al*., 2002; Francis *et al*., 2005). Quantitatively, aldehydes are a main group of compounds in the volatile profile of milk and some of them seem to be characteristic of milk from specific animal species (Moio *et al*., 1993b, 1996; Toso *et al*., 2002). For example, 3-methylbutanal was found only in buffalo milk whereas benzaldehyde and phenylacetaldehyde were not present in caprine milk (Moio *et al*., 1993b). Similarly, the importance of aldehydes in fresh milk flavor differs from one species to another. Nonanal is considered a key compound only in the aroma of raw buffalo milk (Moio *et al*., 1993a) and heptanal makes a special contribution to the aroma of ewes' milk (Moio *et al*., 1996). Differences in the levels of aldehydes have been observed from milks produced by sheep and cows subjected to different diets (Moio *et al*., 1996; Toso *et al*., 2002). In fact, aldehydes provided one of the best discriminant criteria for grouping milks according to type of forage in the ration (Toso *et al*., 2002), which can be very important in identification and validation of origin of dairy products under European protection law.

At low concentrations (approximately 10–40 ppb), aldehydes are associated with pleasant herbaceous notes, but at higher concentrations they can give a penetrating and unpleasant off-flavor (Moio *et al*., 1996). In particular, the contribution of hept-*cis*-4-enal to milk flavor has been investigated. On the basis of its low odor threshold value, its level found in milk (approximately 50 pg/g) and its odor quality has been recognized as an important odorant (Bendall & Olney, 2001).

Unlike the aldehydes, ketones have been largely reported as minor compounds in the raw milk of different ruminants (Moio *et al*., 1993b, 1996). However, other studies have pointed to the ketones as a quantitatively important group in bovine milk (Contarini *et al*., 1997; Toso *et al*., 2002). Different results can be explained by the application of different extraction techniques; however, evidence that ketones are important contributors to fresh milk flavor has not been provided so far. Among ketones, diacetyl has attracted interest because of its typical buttery notes. The role of this compound on overall aroma has not been elucidated as information about its level in unfermented milks is scarce and contradictory. For example, Scanlan *et al*. (1968) reported a concentration of diacetyl in raw milk below threshold value whereas Macciola *et al*. (2008) detected the presence of significant amounts.

Ketones and aldehydes in milk have more than one origin. Methyl ketones with low molecular weight such as propanone and butanone mainly result from the cow's metabolism. Milk fat contains fatty acids which are precursors of methyl ketones with odd-number carbon atoms such as 2-pentanone and 2-heptanone. A mechanism of β-oxidation and decarboxylation of saturated fatty acids or decarboxylation of β-ketoacids naturally present in milk has been proposed (Contarini *et al*., 1997). Diacetyl could be transferred to the milk from forage eaten by the cow (Scanlan *et al*., 1968), directly produced during lactation or it could be formed by enzymatic reaction of certain milk compounds (Macciola *et al*., 2008). Saturated and unsaturated straight-chain aldehydes can be formed by autoxidation of unsaturated fatty acids (Contarini *et al*., 1997). Branched-chain aldehydes and aromatic aldehydes can derive from amino acid catabolism by enzymatic activity (Moio *et al*., 1993b).

Alcohols have been found in small quantities in fresh milk. Their prevalence varies among species, from 1.5% of neutral volatile fraction in bovine milk to 5% in buffalo milk (Moio *et al*., 1993b). Primary and branched-chain alcohols are the main alcohols in milk; they are probably derived from the respective aldehydes by enzymatic reduction (Imhof & Bosset, 1994; Toso *et al*., 2002). During storage of raw milk at refrigeration temperatures, an increased level of alcohols attributed to reduction of carbonyl compounds has been observed (Urbach, 1990b).

Alcohols occur in low quantities in fresh milk and their relative contribution to flavor is considered secondary (Toso *et al*., 2002). Only the 1-octen-3-ol, derived from unsaturated fatty acid degradation, was identified as key odorant in buffalo and ovine milk (Moio *et al*., 1993a).

Esters have been reported as the main group of the neutral volatile fraction of milks from different species (Moio *et al*., 1993b). Ethyl butanoate and ethyl hexanoate are among the most powerful flavor compounds in freshly secreted bovine, caprine, and ovine milk (Moio *et al*., 1993a), which confer a fruity note to the milk (Moio *et al*., 1996). In contrast, a secondary role was observed for esters in pasteurized milk, probably due to their destruction during thermal processes (Moio *et al*., 1994). The origin of esters in raw milk is not fully elucidated. A biosynthesis within the mammary gland or after milking by bacterial activity is believed possible (Moio *et al*., 1993b; Toso *et al*., 2002).

Sulfur and nitrogen compounds are also characteristic compounds of raw and processed milk. Among them, hydrogen sulfide, methanethiol, dimethyl sulfide, carbon disulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl sulfone, 2-acetyl-1-pyrroline, benzothiazole, 2-isobutyl-3-metoxypyrazine, indole, and skatole can

be mentioned (Moio *et al*., 1993b, 1996; Imhof & Bosset, 1994; Al-Attabi *et al*., 2008).

Dimethyl sulfone, a typical sulfur compound, has been identified as a major odorant from a quantitative viewpoint in milk from different mammals, with 25% of the volatile fraction in bovine, caprine, and ovine milk, and only 4% in buffalo milk (Moio *et al*., 1993b). Dimethyl sulfone aroma is described as hot milk, leather, and bovine sweat-like (Vazquez-Landaverde *et al*., 2006a), and its role on milk flavor is controversial: data on threshold values are lacking and wide variations of concentrations have been reported for different diets or thermal treatments (Moio *et al*., 1993a, b, 1994, 1996). On the other hand, dimethyl sulfide, dimethyl trisulfide and methanethiol, which occur at low concentrations, are known to be powerful flavor compounds in milk (Vazquez-Landaverde *et al*., 2005, 2006a).

The presence of sulfur-containing compounds in milk is a consequence of feed given to ruminants but they can also be formed from protein-bound cysteine and methionine, being β-lactoglobulin the main protein involved (Vazquez-Landaverde *et al*., 2006a; de Wit & Nieuwenhuijse, 2008). Catabolism of methionine produces dimethyl sulfide and methanethiol. Methanethiol can be further oxidized to dimethyl disulfide and dimethyl trisulfide (Bendall, 2001; Vazquez-Landaverde *et al*., 2006a). Dimethyl sulfone is probably produced from dimethyl sulfide oxidation or as a result of the cow's metabolism (Moio *et al*., 1993b).

Nitrogen compounds appear to be essential for the aroma of different raw milks. The quantities reported are higher in ovine, caprine, and buffalo milk than in bovine milk, and they can vary widely according to the feed (Moio *et al*., 1993b, 1996). In particular, indole is recognized for its odorant properties: it has a low threshold value and can contribute to milk aroma (Moio *et al*., 1993a, b). At very low levels indole is characterized by a delicate smell reminiscent of jasmine, whereas at higher concentrations it is associated with unpleasant notes of feces (Moio *et al*., 1996).

Several free fatty acids, mainly linear-chain fatty acids of even carbon atoms from C4:0 to C10:0 have been identified in bovine fresh milk, but they are of minor relevance to flavor (Scanlan *et al*., 1965). In contrast, short- and medium-chain fatty acids (C6:0 to C12:0) are the main contributors to sheep and goats' milk flavor and specifically, branched-chain fatty acids are responsible for the characteristic waxy and animal notes (Carunchia Whetstine & Drake, 2006).

Although certain fatty acids can result from amino acid catabolism or lactose degradation, most are derived from triglyceride hydrolysis by lipolytic enzymes. Thus, the particular milk fat composition and lipolytic activities that characterize milk of each ruminant species determines the lipolysis extent and the type of released fatty acid, which in turn impacts greatly on flavor. Little or no lipolysis should occur in fresh milk (Escobar & Bradley, 1990). Therefore, the levels of free fatty acids should be below the detection threshold of rancid off-flavor.

Terpenes, hydrocarbons, and phenolic compounds are normal constituents of fresh milk. Certain terpenes and hydrocarbons are related to diet: it is well-known that monoterpenes and sesquiterpenes can be readily transferred from forages into milk fat, with only minor changes (Viallon *et al*., 2000). Terpenes are products of secondary metabolism of plants, recognized for their disinfectant (medicinal) and odorant (spice) properties. Natural highland pastures rich in dicotyledons generally contain higher quantity and wider diversity of terpenes than the lowland pastures rich in *Gramineae* (Mariaca *et al*., 1997; Viallon *et al*., 2000); they are also more abundant in fresh grass than in hay (Zeppa *et al*., 2004). This profile of terpenes is reflected in milks from cows fed with these pastures and, consequently, terpenic compounds can help to characterize dairy products obtained from milk of ruminants fed different forages, in different seasons, or from different geographical areas (Dumont & Adda, 1978; Viallon *et al*., 2000; Bugaud *et al*., 2001; Zeppa *et al*., 2004). Sesquiterpenes, especially, are considered promising biochemical markers that could be used to link a dairy product to its geographical region (Fernandez *et al*., 2003; Tornambé *et al*., 2006; Povolo *et al*., 2007; Abilleira *et al*., 2011). Unlike the hydrocarbons which probably do not contribute to aroma due to the low concentrations in milk and the high perception thresholds (Moio *et al*., 1993b), terpenes can influence the milk flavor since they are often characterized by a fruity, herbaceous or resinous odor (Addis *et al*., 2006).

A wide variety of phenolic compounds, such as phenol, *o*-, *m*-, and *p*-cresol, 2- and 4-ethyl phenol, thymol, and carvacrol, have been identified in fresh milk (Lopez & Lindsay, 1993; Moio *et al*., 1993b, 1996; Bendall, 2001). A high proportion of them is in the form of metabolic conjugates (glucuronides, sulfates and phosphates) (Lopez & Lindsay, 1993; Kilic & Lindsay, 2005). Qualitative and quantitative differences in the profile of phenolic compounds of milk from different ruminant species have been reported. Phenols in sheep milk were mostly bound as phosphate and sulfate conjugates while in cows' and goats' milk they were mainly bound as sulfates. Levels of *p*-cresol and *m*-cresol were higher in sheep than in goats' and cows' milk but goats' milk contained an exceptionally high concentration of phenol. These alkyl phenols were present as glucuronide and sulfate acid conjugates in all milks (Lopez & Lindsay, 1993). Most phenolic compounds derive from the feed but other possible sources are from amino acid catabolism by bacteria and contamination with

sanitizing agents (O'Connell & Fox, 2001). Although very little is known on the effect of diet on the content of non-volatile phenolic substances in milk, recent research has shown the accumulation of phenolic compounds in the milk of grazing goats (Silanikove *et al*., 2010). At low levels, phenolic compounds may impart desirable sweet, smoky, or caramel notes, but at high levels they can cause off-flavors such as sharp or medicinal (O'Connell & Fox, 2001).

15.3.2 Variations in flavor of fresh milk from ruminant species

Milk is a natural food, biologically produced and, therefore, changes in its flavor are expected (Thomas, 1981). In raw milk, these changes are mainly related to the dairy cattle management system (genetic, physiology, and feeding) and environmental hygiene conditions at the farm level. Variations in milk flavor seem to be caused by concentration differences of a common set of volatile compounds rather than by the occurrence of certain compounds uniquely associated with a particular feed (Bendall, 2001; Mounchili *et al*., 2005). Milking, collection, and storage of raw milk on farms, processing and storage in dairy industries, and displaying and stocking in markets can also modify the characteristic volatile profile of fresh milk.

Aspects related to milk flavor variability are of enormous relevance for the industry as they can lead to serious defects in dairy products. For this reason, the main sources of variations in fresh milk flavor are discussed below.

15.3.2.1 Variations in milk flavor associated with farm management

It is generally agreed that the manufacture of high-quality dairy products begins at the farm level. Milk quality is affected by genetic and physiological characteristics of the ruminants, and the milking systems, storage, and collection of the milk, hygienic conditions of the milking equipment, the farm workers, and the farm overall (McGilliard & Freeman, 1972; Manfredini & Massari, 1989; Coulon *et al*., 2004). Also diets and feeding systems chosen and practiced by the farmer have been shown to influence the sensory quality of milk (Morand-Fehr *et al*., 2007; Butler *et al*., 2011; Zervas & Tsiplakou, 2011). In particular, small ruminant milk flavor is highly influenced by season, diet, milking and cooling practices, and the barn environment (Moio *et al*., 1996; Carunchia Whetstine & Drake, 2006).

15.3.2.1.1 Genetic and physiological characteristics

Breed and other genetic traits of the milking animals, besides age, health status, and lactation stage have important influences on milk flavor. Early studies reported that genetic differences and lactation stage were important sources of variations in milk flavor, whereas the cow's age at calving had little effect on flavor characteristics (Kratzer *et al*., 1967; McGilliard & Freeman, 1972). Studies conducted on goats' milk have indicated that a strong goat flavor can be attributed to milk produced in the middle of lactation with low fat content, high somatic cells counts, and free fatty acid levels compared with herd milk with weak goat flavor intensity (Skjevdal, 1979; Jaubert *et al*., 1996).

15.3.2.1.2 Feeding systems and diets

There is a wide range of feeding systems all over the world and they can be grouped in different types. The two major systems are pasture and indoor feeding, which have variable levels of intensification from very extensive to very intensive (Zervas & Tsiplakou, 2011). Feed types (such as forages, concentrates, by-products), forage preservation methods (hay or silage), pasture grass botanical composition, and different types of diets have been extensively studied as a medium to increase either the unsaturated fatty acid levels (especially conjugated linoleic acid, CLA) or the antioxidative capacity of milk. In studies of the relationship between diets given to ruminants and free fatty acid composition of milk fat it has been shown that a high content of unsaturated fatty acids in milk fat may not necessarily be positive for milk quality since the double bonds of fatty acids can promote defects, mainly via oxidative reactions. For this reason, the oxidative stability of milk is still a topic of concern to the dairy industry. Among several implications for milk quality, a shorter shelf-life, off-flavor development (e.g., oxidized, grassy and cowy), and nutritional deterioration can occur (Havemose *et al*., 2004, 2006). Also, specific off-flavors in milk have been attributed to specific diet components, and the compounds responsible have been identified (Urbach, 1990a).

Defects produced by free fatty acid oxidation

The oxidative stability of milk results from a delicate balance between antioxidant and pro-oxidant activities, influenced in turn by several factors such as composition of fatty acids, transition metal ions, and antioxidants (Smet *et al*., 2008). Manipulation of feeding can increase the content of unsaturated fatty acids and make the milk more susceptible to oxidation, or improve the oxidative stability of milk if antioxidants can be transferred from feed to milk (Havemose *et al*., 2004). Numerous studies have focused on sensory characteristics of milk naturally enriched with polyunsaturated fatty acids, mainly CLA. It has been suggested that pasture-based (PB) production systems may contribute to health benefits for the consumer due to the presence of higher concentrations of CLA than when cows were fed concentrates, silages, or conventional total mixed

rations (TMRs) (White *et al*., 2001; Elgersma *et al*., 2006; Butler *et al*., 2011). Milk obtained from PB and TMR systems showed some differences in the volatile profile and overall flavor (Bendall, 2001; Croissant *et al*., 2007), but whereas trained panelists reported greater intensities of grassy and cowy/barny flavors in PB milk than in TMR milk, consumers were unable to differentiate them (Croissant *et al*., 2007). Other studies have considered the effects of diets with different ratios of red clover and grass silage on the volatile profile or sensory characteristics of milk, since the incorporation of red clover silage leads to increased levels of polyunsaturated fatty acids. Results obtained so far are contradictory. Al-Mabruk *et al*. (2004) reported a decrease in oxidative stability during the storage of milk produced from red clover silage compared with milk from diets based on grass silage. According to Moorby *et al*. (2009) there were no effects on the aroma or overall flavor of milk when the proportion of red clover in the diet of dairy cows was increased. Oxidative stability of milk enriched with natural CLA through diets supplemented with soybean oil and fish oil has been also evaluated. Lynch *et al*. (2005) analyzed both flavor and stability of pasteurized milk with and without naturally increased levels of CLA. Untrained panelists were unable to detect flavor differences during the storage period and, thus, sensory analysis indicated no difference in susceptibility to the development of oxidized off-flavors between both types of milks. Most research carried out on sensory characteristics of milk enriched with CLA has obtained the same results (Baer *et al*., 2001; Ramaswamy *et al*., 2001a, b; Nelson & Martini, 2009), which suggests that the levels of increased CLA do not promote the oxidative deterioration of milk.

The effect of antioxidants and pro-oxidants on oxidative stability of milk has also been evaluated, since it was suggested that oxidized off-flavor could be inhibited by the presence of certain antioxidants such as α-tocopherol, β-carotene, ascorbic acid, and selenium or enhanced by the action of certain pro-oxidants such as copper (Barrefors *et al*., 1995; van Aardt *et al*., 2005a; Havemose *et al*., 2006). Milk with high unsaturated fatty acid levels is most susceptible to oxidation, especially when it has high concentrations of copper (Timmons *et al*., 2001). Natural (tocopherols, ascorbic acid, etc.) and synthetic (butylated hydroxyanisole, butylated hydroxytoluene, etc.) antioxidants have attracted much attention as a medium of preventing or delaying oxidation. Milk contains low concentrations of natural antioxidants, which also depend on feeding, but they can only initially avoid oxidative deterioration (van Aardt *et al*., 2005a). The effect of antioxidants on the oxidative stability is highly dependent on their concentrations and on the level of polyunsaturated fatty acids in milk. For similar concentrations of natural antioxidants, raw milk containing more unsaturated fatty acids was more sensitive to oxidation (Hedegaard *et al*., 2006). According to Havemose *et al*. (2004, 2006), the role of natural antioxidants to prevent lipid oxidation seems to be less important than the fatty acid composition. By contrast, milk supplementation with natural or synthetic antioxidants was effective against oxidized off-flavor during storage (Al-Mabruk *et al*., 2004; van Aardt *et al*., 2005a, b). Recently, oxidative stability of milk was evaluated in relation to selenium supplementation to the cow's diet (Clausen *et al*., 2010). The results indicated that this natural antioxidant has no effect on oxidative stability of milk.

Among the tools available to determine the oxidative stability of milk as a function of time, the measure of certain primary and secondary oxidation products might to be a good parameter (Hedegaard *et al*., 2006). Oxidative off-flavor is closely related to increased concentrations of saturated straight-chain aldehydes from C5 to C9 (Jenq *et al*., 1988; Barrefors *et al*., 1995). In particular, the assessment of hexanal correlate well with oxidized sensory descriptors (Hedegaard *et al*., 2006). Similarly, sulfur-containing compounds (dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide), alkenals (2-octenal, 2-heptenal, 2-nonenal), alkadienals (2,4-nonadienal, 2,4-decadienal, etc.), unsaturated ketones (1-octen-3-one), and alcohols (1-octen-3-ol) have also been reported as typical compounds that impart oxidized off-flavors to dairy products (Barrefors *et al*., 1995; van Aardt *et al*., 2005a, b; Marsili, 2011).

Defects related to specific feeds

Feeding of the dairy herd can affect not only fatty acid composition of milk fat and oxidative stability of milk. Certain diets and grass botanical composition can affect the volatile profile by modifying the quantities of some compounds that belong to chemical groups such as terpenes (Mariaca *et al*., 1997; Bugaud *et al*., 2001; Addis *et al*., 2006; Tornambé *et al*., 2006), hydrocarbons (Addis *et al*., 2006), aldehydes (Moio *et al*., 1996; Bendall, 2001; Toso *et al*., 2002), lactones (Bendall, 2001), sulfur and nitrogen compounds (Moio *et al*., 1996; Bendall, 2001), and phenolic compounds (Silanikove *et al*., 2010), which not necessarily lead to reduced sensory quality of milk. However, certain fodder plants and silages consumed by cows such as fermented musty silage (maize, legumes, and grass), sugar beet by-products, fruit and vegetable residues, alfalfa (green or hay), clover hay, onion weeds, and green barley have been linked to negative sensory attributes, giving milk some undesirable notes such as fishy, onion, silage, herb-like, green grass (Marsili, 2011). This topic has been extensively reviewed by Urbach (1990a) and therefore only some examples are mentioned. Trimethylamine

and methyl sulfide were largely reported as responsible for the fishy and malty/cowy flavors of milks from cows fed wheat pasture and freshly cut alfalfa, respectively (Reddy *et al*., 1966; Mehta *et al*., 1974). A specific rancid and tart taste, probably due to lipolysis of milk fat, is the most common defect in Norwegian goats' milk during some seasons, whose frequency has been related to certain types and levels of concentrates and roughage (Eknaes & Skeie, 2006; Eknaes *et al*., 2009). Silages have been specifically investigated as a potential source of feedy or silage notes. Silages that are not well fermented are most commonly responsible for imparting this adverse taste to milk. The term "feedy" is used to describe a variety of flavors having similar characteristics. In early studies it was reported that acetone, 2-butanone, dimethyl sulfide and *cis*-3-hexen-1-ol caused tainted milk by feedy flavors (Shipe *et al*., 1962). Recently, 2-butanone and dimethyl sulfide (and to some extent ethanol and 2-propanone) were suggested as probable markers for this defect (Mounchili *et al*., 2005).

15.3.2.1.3 Environmental conditions

Studies on the causes of flavor changes in goat milk have shown that unclean handling of the milk reduces the genuine flavor (Skjevdal, 1979). In an atmosphere dominated by silage or animal odors, volatile compounds may be transferred directly from the surrounding environment to the milk. Transmitted or absorbed off-flavors can occur in milk before, during, and after milking. The odor of the male goat in rut is often implicated as a source of the goaty flavor problem in fresh goats' milk. The high volatility of buck odors has led to a recommended practice on farms of isolating the buck some distance from the milking area; 6-*trans*-nonenal generated by the sebaceous gland of the scalps of sexually active bucks as a pheromone or as a product from oxidation of certain precursors in the gland lipids was identified as a major contributor to this odor (Smith *et al*., 1984).

15.3.2.1.4 Raw milk quality and milk manipulation

The main causes of flavor variations in raw milk quality are from milk manipulation related to the activity of enzymes in the milk, spoilage microorganisms or somatic cells, and off-flavors produced by lipolysis and proteolysis are the most common.

Defects caused by induced and spontaneous lipolysis

Hydrolytic rancidity of milk lipids or lipolysis is perhaps the main defect observed in farm milk. This defect must be distinguished from the oxidative rancidity (Deeth, 2011) resulting from lipid oxidation. The hydrolysis of triglycerides, the major constituents of milk fat, is catalyzed by lipases producing free fatty acids, some of which have low perception thresholds and unpleasant aromatic notes (rancid, butyric, bitter, unclean, soapy, or astringent) (Deeth & Fitz-Gerald, 2006; Deeth, 2011). Endogenous milk lipase or lipoprotein lipase (LPL) is one of the main types of enzymes responsible for this reaction. The natural lipolytic system and fatty acid composition of milk fat differ considerably between ruminant species (Chilliard *et al*., 2003). Lipase activity in ovine milk is about one-tenth and in goats' milk is about one-third than that of bovine milk. Goats' milk fat has significantly higher levels of short- and medium-chain linear fatty acids (C4:0 to C14:0) and branched-chain fatty acids than cows' milk (Park 2006). Owing to the specificity of the LPL, the hydrolysis patterns of milk fat are also different (Albenzio & Santillo, 2011). It has been reported that linear short-chain fatty acids contribute markedly to rancid off-flavors in bovine milk (Scanlan *et al*., 1965; González-Córdova & Vallejo-Cordoba, 2001), medium-chain fatty acids can be responsible for this defect in ovine milk (Albenzio & Santillo, 2011), whereas in goats' milk rancidity is due to free fatty acids from C6:0 to C9:0 and more specifically from volatile branched-chain acids such as 4-methyl and 4-ethyl octanoic acids (Eknaes *et al*., 2009).

In raw milk, little or no lipolysis should occur, as the lipase usually cannot make contact with the substrate, since the triacylglycerols are protected in globules covered by a membrane (Escobar & Bradley, 1990). Rancidity is caused by weakened or broken milk fat globule membranes, which is promoted by several factors. Two types of lipolysis seem be related to the action of native lipases of milk of good milk quality: induced and spontaneous.

Induced lipolysis is related to the damage of the globule membrane caused by pumping, mechanical agitation, foaming, mixing of air into the milk, milking systems, temperature changes, slow cooling, freezing and thawing in storage (Escobar & Bradley, 1990; Slaghuis *et al*., 2004; Deeth, 2011). Certain management practices on the farm, including the use of modern but improperly designed milking systems, can induce rancidity. Automatic robot milking systems can lead to milk with higher free fatty acid levels than that obtained in a conventional milking parlor (Abeni *et al*., 2005). Inadequate maintenance, faulty design and installation of milking machines, teat cups, and pipelines can produce excessive air intake with the milk, causing turbulence and frothing of the milk, and thus damage to the fat globule membrane (Slaghuis *et al*., 2004; Deeth, 2011). High pipeline milking systems, lifting milk by air in vertical pipe sections, especially with a high air to milk flow ratio, and high speed of pumping of milk to the bulk tank are some design features that promote increased levels of lipolysis (Escobar & Bradley, 1990; Slaghuis *et al*., 2004).

Spontaneous lipolysis is defined as that which occurs in some individual milk when cooled soon after milking without any other treatment. Individual cows differ in the susceptibility of their milk to spontaneous lipolysis (Escobar & Bradley, 1990; Deeth & Fitz-Gerald, 2006). This phenomenon is observed most often when a large number of cows are in late lactation and/or when good-quality feed is not available (Deeth, 2011). In this case, the fat globule is probably not disrupted, but several factors favor the interaction of LPL with milk fat (Slaghuis *et al*., 2004). Some management practices on farms such as milking frequencies, generally associated with automatic milking, apparently lead to increased free fatty acid levels (Abeni *et al*., 2005). However, one of the main factors influencing spontaneous lipolysis is the activator/inhibitor ratio (Cartier & Chilliard, 1990). In bovine milk, lipolysis remains generally low despite the high LPL activity. This could be due to inhibitors and to the fact that in bovine milk LPL is largely bound to casein micelles, compartmentalized from the substrate. In contrast, large proportions of LPL are bound to cream in goat milk, which could explain its higher sensitivity to spontaneous lipolysis. In goat milk the lipolysis level correlates well to LPL activity (Chilliard *et al*., 2003; Park, 2006).

Defects caused by microorganism growth

Psychrotrophic bacteria are the dominant type of bacterial flora in raw milk after a period of refrigerated storage (Deeth, 2011). They multiply at low temperatures and may have negative effects to lipolysis and proteolysis if the storage is prolonged (Manfredini & Massari, 1989; Celestino *et al*., 1996). Rancid, unclean, soapy, cardboard, oxidized, and metallic flavors are typical defects produced by lipases of psychrotrophic bacteria via lipolysis (Champagne *et al*., 1994). In addition, putrid flavor caused by proteolysis is the result of bacterial contamination, holding raw milk for 3 or 4 days after collection, and storage temperature above 5°C (40°F) (Dairy Practices Council, 1991).

Defects caused by high somatic cell counts

Another important source of flavor variation, closely linked to the bacteriological quality of the milk, is the somatic cell count (SCC). SCC is dependent on ruminant species and dairy herd management (breed, stage of lactation, season, udder health, etc.) (Albenzio & Santillo, 2011), and it is considered a good index of the hygienic and sanitary aspects of the milk (Raynal-Ljutovac *et al*., 2007). Both bacterial load and SCC can be diminished by improved management conditions, which include sanitation at the farm of animals and milking facility, udder cleaning and teat sealing, milking equipment maintenance, and timely transfer to the refrigerated tank (Goetsch *et al*., 2011). Increased SCC is correlated with increased levels of proteolysis and lipolysis and a higher activity of heat-stable protease (plasmin) and lipase (LPL) enzymes even during refrigerated storage of raw and pasteurized milk (Senyk *et al*., 1985; Ma *et al*., 2000; Barbano *et al*., 2006; Gargouri *et al*., 2008). Main sensory defects related to high SCC include bitterness and rancidity (Ma *et al*., 2000). Hydrophobic peptides such as those derived from β-casein are related to the appearance of bitterness (Harwalkar *et al*., 1993), while lipolysis causes rancid off-flavor (Raynal-Ljutovac *et al*., 2007). Bovine milk with high SCC, pasteurized and stored refrigerated, developed rancidity as the predominant flavor defect between 14 and 21 days (Ma *et al*., 2000), but proteolysis may produce off-flavor earlier during shelf-life than lipolysis (Barbano *et al*., 2006). Concerning small ruminant milk, increased goaty flavor and lipolytic activity were partly associated with higher levels of SCC (Jaubert *et al*., 1996). Proteolysis, plasmin activity and SCC were positively correlated in goats' and ewes' milk (Raynal-Ljutovac *et al*., 2007).

Lipolysis and proteolysis thresholds

Off-flavors caused by hydrolysis of triglycerides and of proteins by proteases or lipases from somatic cells, endogenous in milk or of bacterial origin, are severe drawbacks for the quality of dairy products and therefore their sensory thresholds have been assessed. Traditionally, the acid degree value (ADV) has been used as a measure of hydrolytic rancidity (González-Córdova & Vallejo-Cordoba, 2001). According to this index, the threshold for lipolyzed flavor detection in milk is within the range 4.1–4.5 mEq KOH/100 g fat (Pillay *et al*., 1980), or approximately 1.0 mEq FFA/100 g fat (Ma *et al*., 2000). More recently, the sensory threshold for lipolysis in milk with a fat content of 2% was determined by an ascending forced-choice procedure, with a series of triangle tests in different sessions. In this study, the lipolysis detection threshold was established between 0.32 and 0.35 mEq of free fatty acid per kilogram of milk (Santos *et al*., 2003), whereas the sensory threshold of bitterness by proteolysis in milk with 2% fat and skim milk was equivalent to a decrease of 4% and 4.8% of the casein, respectively (Ma *et al*., 2000; Santos *et al*., 2003).

15.3.2.2 Variations in milk flavor associated with factory management

Milk industrialization influences the natural fresh milk flavor. Depending on the industrial practices, some changes can be detrimental. Among the potential sources of off-flavor are manipulation and storage of raw milk, severity of thermal treatment and storage conditions of processed milk including packaging.

15.3.2.2.1 Manipulation and storage of raw milk

Owing to changes in milk collection from farms and management practices at dairies, some fluid milk plants may be processing raw milk as old as 5 days (Champagne *et al*., 1994). In dairy plants, certain conditions that promote lipolysis and psychrotrophic bacteria growth can take place. These are failures to empty and wash raw milk from tanks, air leaks in pipes, excessive pumping, homogenization without immediate pasteurization, and mixing of homogenized and raw milk (Dairy Practices Council, 1991; Deeth, 2011).

Defects caused by lipolysis in pasteurized milk are the same as those produced in raw milk. The dominant species limiting the shelf-life of refrigerated fluid milk is *Pseudomonas* spp. (Dogan & Boor, 2003). Psychrotrophs are destroyed by thermal treatments but their enzymes are thermostable. In this case, *Pseudomonas* spp. need to grow at relatively high levels in raw milk before pasteurization to produce verifiable defects in processed milk at the end of shelf-life (Barbano *et al*., 2006). Furthermore, they may contaminate milk after pasteurization if the pumping, holding, and filling system is not properly cleaned and sanitized.

Undesirable flavors due to microbial growth include bitter, rancid, fruity, and unclean (Champagne *et al*., 1994). *Pseudomonas fragi* is recognized as the microorganism responsible for the production of a fruity or strawberry-like odor, with ethyl butanoate, ethyl hexanoate and ethyl-3-methyl butanoate the major contributors (Cormier *et al*., 1991). Another microbial-induced off-flavor is a malty aroma due to methyl aldehydes and methyl alcohols such as 2-methylbutanal, 3-methylbutanal, 2-methyl-1-butanol, and 3-methyl-1-butanol (Marsili, 2011). *Lactococcus lactis* var. *maltigenes* is the main microorganism responsible for this defect, which is related to poor refrigeration of milk.

15.3.2.2.2 Severity of thermal treatment

More rigorous thermal treatments than that strictly needed for pasteurization can be an effective approach to extend milk shelf-life, and is applied on many occasions, depending on the dairy plant and the country. However, severe heat treatment can cause negative sensory attributes and consequently a decrease in the acceptability of milk by consumers. This topic was recently investigated by Gandy *et al*. (2008), who analyzed the effect of four pasteurization temperatures (77, 79, 82, and 85°C/15 s) on consumer acceptability, sensory characteristics, and shelf-life of fluid milk. This study revealed that milk processed up to 79°C was highly acceptable to all consumers. This research also showed that milk samples could not be differentiated based on pasteurization temperature when tested toward the end of shelf-life, suggesting that sensory differences evened out as storage time elapsed.

15.3.2.2.3 Storage conditions of processed milk and packaging type

Milk flavor can be strongly altered as a consequence of the storage conditions. The packaging material determines largely the degree to which certain physical agents can act, promoting undesirable reactions (Moyssiadi *et al*., 2004). An undesirable flavor known as packaging can be induced by contact of milk with certain types of packaging materials.

Packaging material can maintain the pasteurized milk quality by controlling oxygen permeability and light transmission as well as by providing perfect seals to avoid microbial recontaminations (Vassila *et al*., 2002). In addition to the traditional glass bottles and coated paperboard cartons, several plastic containers, such as polyethylene terephthalate (PET) and high-density polyethylene (HDPE) available as clear, pigmented, monolayer, and multilayer bottles, are commonly used in pasteurized milk packaging. Moreover, polyethylene pouches in the form of flexible monolayer or low-density polyethylene (LDPE) and multilayer coextruded pouches based on LDPE are also found in the market (Vassila *et al*., 2002; Zygoura *et al*., 2004).

Oxidation in processed dairy products is due to acceleration of reactions already initiated in the raw milk (Hedegaard *et al*., 2006). The two main mechanisms of milk oxidation are the oxidation induced by certain metals or autoxidation, clearly prevalent in light-protected milk, and oxidation induced by light (Smet *et al*., 2008). Different patterns of flavor deterioration are observed in each case (Karatapanis *et al*., 2006).

Light-oxidized off-flavor can develop when fresh milk is packaged in light-transmissible containers and then stored in lighted displays (Chapman *et al*., 2002). It has been estimated that exposure to light of milk packaged in HDPE containers and exposed in dairy cabinets is responsible for light-induced flavor defects in 80% of samples sold in supermarkets in USA (Marsili, 2011). Light exposure can also adversely affect the nutritional value of milk producing vitamin loss (Olsen & Ashoor, 1987; Lee *et al*., 1998; Whited *et al*., 2002). Both light and riboflavin participate in photo-induced chemical reactions that involve certain fatty acids and amino acids as substrates (Kim & Morr, 1996). Typical descriptors related to the oxidation processes include old vegetable oil, cardboard, goat, or metallic (Chapman *et al*., 2002; Hedegaard *et al*., 2006). The presence of methional, an initial product of methionine degradation, is directly related to exposure of milk to light (Allen & Parks, 1975). Other volatile compounds such as methanethiol, dimethyl sulfide, dimethyl disulfide, pentanal, hexanal, 2-butanone, and 1-octen-3-one have been also identified as light-activated flavors (Jenq *et al*., 1988; Kim & Morr, 1996; Karatapanis *et al*., 2006; Marsili, 2011). This defect is rapidly perceived by trained panelist

and consumers. Sensory thresholds of light-oxidized off-flavors were determined by Chapman *et al*. (2001). According to these authors trained panelist were able to detect defects in reduced fat milk packaged in HDPE containers after 15–30 min of exposure to fluorescent light while consumers detected light-induced off-flavors after 54 min to 2 hours.

Numerous studies have compared the effect of several packaging materials on keeping quality (defined as the number of days between manufacture and spoilage) of pasteurized milk by determining lipid oxidation, induced-light oxidation, vitamin degradation, proteolysis, lipolysis, and microbial growth. Bottles made from PET incorporating UV blocking agents (Cladman *et al*., 1998; van Aardt *et al*., 2001; Papachristou *et al*., 2006) and multilayer pigmented HDPE (Moyssiadi *et al*., 2004; Zygoura *et al*., 2004; Karatapanis *et al*., 2006), coextruded pouches based on LDPE containing both white and black pigments and thickness above 100 μm (Vassila *et al*., 2002) and laminated cartons with ethylene-vinyl alcohol and aluminum foil (Simon & Hansen, 2001b) have been reported as packaging that provides the best overall protection for the pasteurized milk, being attractive and convenient alternatives to the coated paperboard cartons.

As for the packaging off-flavor, its presence was detected in milk induced by contact with PE film and PE-coated paper. The defect is easier to perceive in skim milk, and container and the area/volume ratio of the package is important for its development, while the type of heat-sealing does not appear to influence it. The off-flavor appears at the beginning of the storage and does not increase with time (Leong *et al*., 1992).

15.3.3 Volatile profile and sensory characteristics of heat-treated milk

Thermal processes alter the sensory characteristics of milk. The kind and intensity of this change is related to the time and temperature of the treatment (Scanlan *et al*., 1968; Shipe *et al*., 1978). The appearance of heat-induced flavors is inevitable in any heated milk, but mainly in those treated with more rigorous temperature–time conditions, such as UP, UHT processes, concentration, in-container sterilization, and drying than conventional pasteurization. As the intensity of thermal treatment increases, the levels of volatile compounds derived from proteins, carbohydrates, and lipids also are augmented, and the heat-induced flavors are more strongly detected (Badings & Neeter, 1980; Calvo & de la Hoz, 1992). Thus, the typical flavor of fresh UHT milk is described as cooked or cabbagey, whereas flavor of sterilized and concentrated milk is characterized by caramelized or burnt notes (Nursten, 1997). Certain indexes can give information about the thermal history of a milk sample, namely the degree of whey protein denaturation and the presence of compounds from Maillard reactions (Guingamp *et al*., 1999) or the levels of a reduced set of volatile compounds thermally formed (Contarini *et al*., 1997).

15.3.3.1 Ultrapasteurized milk and ultra-high-temperature treated milk

The sensory characteristics of UP milk must be similar to those of pasteurized milk (Simon & Hansen, 2001a) and therefore attributes of heated milk should be slightly perceived. Chapman *et al*. (2001) described the key sensory characteristics of UP milk samples of different fat levels using quantitative descriptive analysis. Intensity of descriptors was higher for cooked, sweet, and caramelized notes, indicating their relevance in overall flavor. Flavor development in milk of extended shelf-life is a dynamic process that evolves over time. Cooked notes that characterize fresh UHT and UP milks dissipate after a few days depending on storage temperature (Celestino *et al*., 1997b; Chapman *et al*., 2001), possibly due to loss of volatile sulfides (Simon *et al*., 2001) or by oxidation of sulfhydryl groups (Celestino *et al*., 1997b; Simon & Hansen, 2001a), giving a maximum acceptability after a few days (Nursten, 1997). Then, in a second stage, flavor of UHT or UP milk suffers deterioration and the overall quality declines slowly during storage as milk develops a flavor described as stale, bitter, heated, or sterile (Nursten, 1997; Chapman *et al*., 2001). It has been reported that a slight sweet taste can appear after the cooked flavor has dissipated, which is commonly considered as acceptable (Celestino *et al*., 1997b).

Cooked and cabbagey notes have been attributed to volatile sulfur compounds such as hydrogen sulfide, methanethiol, methyl disulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl sulfone, among others (Al-Attabi *et al*., 2008). As the processing temperature increases, the levels of these compounds increase in parallel (Simon *et al*., 2001; Vazquez-Landaverde *et al*., 2005). The main source of them is the sulfhydryl group of proteins, mainly whey proteins such as β-lactoglobulin and to a minor extent fat globule membrane proteins, which can react during heating (Clare *et al*., 2005). An interesting research about kinetic modeling of the formation of sulfur-containing components during heat-treatment of milk was performed by de Wit & Nieuwenhuijse (2008). According to this study, cooked flavor showed high positive correlations with the concentration of methanethiol.

In spite of its importance on overall flavor, sulfur compounds are not the main volatile components of heated milk from a quantitative viewpoint. Methyl ketones have been reported as the most abundant class of compounds (Jeon *et al*., 1978; Contarini *et al*., 1997; Valero *et al*., 2001; Solano-Lopez *et al*., 2005), and it is well known that

they are produced during thermal treatments (Contarini *et al*., 1997; Vazquez-Landaverde *et al*., 2005). Although a minor role on milk flavor has been reported (Jeon *et al*., 1978), methyl ketones have been extensively studied since their content is related to the intensity of thermal treatment applied to milk (Scanlan *et al*., 1968; Contarini *et al*., 1997). In particular, the concentration of 2-heptanone seems to be a suitable marker for heat treatment (Contarini & Povolo, 2002; Avalli *et al*., 2004).

Aldehydes, lactones, alcohols, nitrogen-containing compounds, esters, and hydrocarbons are other classes of volatile compounds commonly identified in UHT and UP milks (Jeon *et al*., 1978; Simon *et al*., 2001; Valero *et al*., 2001; Solano-Lopez *et al*., 2005). Among them, benzothiazole, δ-lactones, and hexanal have been suggested as typical heat-induced compounds (Scanlan *et al*., 1968; Calvo & de la Hoz, 1992; Contarini *et al*., 1997), making a moderate contribution to the flavor of this type of milk (Moio *et al*., 1994). Unlike in fresh milk, esters have a secondary role on flavor, which has been attributed to their thermal destruction (Moio *et al*., 1994; Marsili, 2011).

15.3.3.2 Milk powder, sterilized, and concentrated milk

Among thermally treated milks, a special consideration has always been given to milk powder or dry milk. Ideally, milk powder should have aromatic notes similar to fluid milk, that is, cooked, sweet, and milk fat free of defects such as grassy or painty flavors (Lloyd *et al*., 2009a). Skim milk powder (SMP) and whole milk powder (WMP) are widely used as food ingredients and for direct consumption (Karagül-Yüceer *et al*., 2001). However, these two types of milk powders have some flavor differences. Studies carried out by Drake *et al*. (2003) on sensory attributes of milk powder have indicated that cooked, sweet aromatic, salty, astringent, cardboard, potato/brothy, cereal, and animal are attributes frequently observed in SMP. Cooked flavors may be present and vary according to heat treatment (low, medium, and heat) of the milk prior to evaporation. The application of principal component analysis to characterize and to distinguish milk powder samples according to their attributes showed some interesting results. SMP samples were grouped into three categories. One group was characterized by sweet aromatic, sweet taste, and cooked, being the most closely associated with the typical flavor of fluid skim milk. Another group was characterized by animal, potato/brothy, and astringent flavor and a few samples were described by cereal/grassy and cardboard notes. On the other hand, attributes reported for WMP were described as cooked, caramelized, sweet aromatic, milk fat, fried, salty, astringent, cereal, among others. Certain flavors such as milk fat, fried and fatty/painty were only detected in WMP, which is in accordance with the fact that these flavors are derived from milk fat.

Volatile compounds present in fresh milk powder have been extensively investigated (Shiratsuchi *et al*., 1994a; Karagül-Yüceer *et al*., 2001, 2002; Lloyd *et al*., 2009a, b). Key aroma compounds identified in SMP were verified by sensory analysis on model mixtures (Karagül-Yüceer *et al*., 2004). From these studies the important contributions were established to the overall flavor for free fatty acids, lactones, and a set of heterocyclic compounds such as furaneol, maltol, and sotolon, and the minor role of hydrocarbons, saturated and non-saturated aldehydes, methyl ketones, and alcohols. The chemical nature of these compounds reveals the key role of milk fat hydrolysis and Maillard reactions, which are chemical processes encouraged by heat. Sweet and milk odors in SMP have been correlated with levels of nonanoic, decanoic, undecanoic, and dodecanoic acids and some δ- and γ-lactones (Shiratsuchi *et al*., 1995).

Flavor of sterilized concentrated milk has been described as caramelized, which intensifies during storage at room temperature. Analysis of volatile compounds has revealed the presence of methyl ketones, benzaldehyde, *o*-aminoacetophenone, 2-furfural, furfuryl alcohol, hydroxymethylfurfural, benzothiazole, fatty acids, and δ-lactones (Arnold & Lindsay, 1969; Loney & Bassette, 1971; Badings & Neeter, 1980). Among them, methyl ketones could contribute to overall flavor (Allen & Parks, 1969; Arnold & Lindsay, 1969). Taking into account that the levels of some of these compounds such as methyl ketones, *o*-aminoacetophenone and free fatty acids may increase during storage and thus generate off-flavor, their evolution through the storage process has also been investigated (Patel *et al*., 1962; Loney & Bassette, 1971).

15.3.3.3 Infant formula

They are substitutes of human milk for infants when a mother cannot breastfeed or chooses not to breastfeed (O'Callaghan *et al*., 2011). Since it was largely suggested that breast milk has a higher acceptance by infants than formulated milks and both seem to differ in flavor, attention has been focused on differences in volatile composition and sensory characteristics between them. Hausner *et al*. (2009) found that the volatile fraction of human breast milk was characterized by aldehydes, terpenes, alcohols, and ketones, whereas carbonyl compounds such as ketones and aldehydes represented the most important class of volatiles in infant formula milk. According to these authors, formulae differed from mothers' milk as they contained some volatiles related to heat treatment such as methional, 2-furfural and sulfides, which were not present in mothers' milk. On the other hand, breast milk had a higher variety of terpenes, aldehydes, and alcohols.

15.3.4 Variations in flavor of heat-treated milk

Heating affects milk quality as a consequence of interactions between amino acid lateral groups, degradation reactions of lateral chains of the proteins, restructuring of SH and S–S groups, whey protein insolubilization, interactions between κ-casein and β-lactoglobulin, interactions of proteins with lipids, and interactions between carbohydrates and proteins, namely the Maillard reaction (Ferrer *et al*., 2000a). From a sensorial viewpoint, the potential sources of undesirable flavors are commonly related to lipid oxidation and Maillard reactions. Flavor defects may also indicate spoilage and microbial growth.

15.3.4.1 Ultrapasteurized milk and ultra-high-temperature treated milk

Oxidized, cardboardy, lack of freshness, fruity, and hammy are the most common off-flavors reported in UP milk (Simon & Hansen, 2001a; Simon *et al*., 2001). Chapman *et al*. (2001) observed that bitter flavor, bitter aftertaste, drying, and lingering aftertaste develop in UP samples at the end of shelf-life, contributing to the reduction in the overall quality rating. These defects were attributed to the enzymatic activity of lipases and proteases that can survive thermal processes. Flavor deterioration in UP milk has not been investigated as extensively as in UHT milk and the few existing research studies have focused on the effects of processing temperature and packaging material on shelf-life and flavor (Simon & Hansen, 2001a; Simon *et al*., 2001; Solano-Lopez *et al*., 2005). Results showed that milk processed at higher temperatures contained the highest relative amounts of sulfur compounds, whose retention in the product depended on the type of barriers and foil boards of the packaging. Hammy, cardboardy, and cooked notes derived from packaging type and their intensity increased with storage time, so that rates of milk flavor deterioration varied according to packaging materials. Rancidity was not reported as a problem during storage of UP milk.

Cooked note in UP milk is minimal compared with regularly processed UHT milk, which can develop heat-induced off-flavors (Solano-Lopez *et al*., 2005). Certain thermally derived compounds such as 2,3-butanedione, 2-heptanone, 2-nonanone, 2-methylpropanal, 3-methylbutanal, nonanal, decanal, and dimethyl sulfide were identified as important contributors to the off-flavor of UHT milk (Vazquez-Landaverde *et al*., 2005). In addition to defects induced by heat, other causes of low sensory quality of stored UHT milk seem to be proteolysis, lipolysis, oxidative reactions, and non-enzymatic browning (Al-Kanhal *et al*., 1994; Valero *et al*., 2001; Gaucher *et al*., 2008).

Enzymatic processes occur as a consequence of the reactivation of heat-resistant enzymes during prolonged storage (Chen *et al*., 2003). Although both proteolysis and lipolysis are considered the most important factors limiting the shelf-life of UHT milk during storage at room temperature through changes in flavor and texture, the main enzymatic defects reported are related to proteolysis. Bitter, unclean, sour, and putrid notes are attributed to the action of plasmin and/or proteinases from spoilage microorganisms of raw milk (Datta & Deeth, 2003). Recommendations on the limits of proteinase activity or tyrosine levels in UHT milks to ensure certain shelf-life have been proposed, but these values are highly dependent on assay techniques (Chen *et al*., 2003).

Stale, metallic, and/or oxidized off-flavor derived from lipid oxidation can be detected during storage of UHT milk with different intensity (Valero *et al*., 2001). Cooked flavors mask or prevent the detection of oxidative defects at the beginning of storage, but as sulfhydryl groups are oxidized and volatile sulfur compounds dissipate, defects can be perceived (Celestino *et al*., 1997b). Concentrations of certain carbonyl compounds, mainly straight-chain aldehydes and methyl ketones have been closely related to staleness (Jeon *et al*., 1978; Rerkrai *et al*., 1987).The contribution of dissolved oxygen, packaging type and storage conditions (time and temperature) on its development has been investigated. In general, staleness is more pronounced at higher temperatures and longer times of storage (Mehta & Bassette, 1980; Al-Kanhal *et al*., 1994; Celestino *et al*., 1997b). The role of oxygen level is controversial. Some authors have reported that a higher initial oxygen content seems to be beneficial to flavor in the early life of the milk due to the reaction with sulfhydryl groups responsible for cooked notes (Nursten, 1997), but after some weeks its effect becomes negligible (Thomas *et al*., 1975). Others have suggested that the presence of oxygen increases the rate of aldehyde formation (Jeon *et al*., 1978) or that it has an unclear influence (Rerkrai *et al*., 1987). Regarding packaging materials, overall flavor of UHT milk protected with aluminum foil (Mehta & Bassette, 1980; Rysstad *et al*., 1998) or packaged in aseptic pouches with oxygen-scavenging film (Perkins *et al*., 2007) and in PET and HDPE bottles with light barrier (Mestdagh *et al*., 2005; Smet *et al*., 2009) was well maintained, and the appearance of staleness was delayed compared with other types of packaging.

Certain markers of the Maillard reaction such as furosine and furfural have been assessed in UHT milk since they are evidence to the use of low-quality raw material, the application of too rigorous conditions of processing, or inadequate storage (Ferrer *et al*., 2000a). Maillard reaction does not give rise to flavor changes in the case of UTH milk treated by conventional processes (van Boekel, 1998).

In reconstituted UHT milk, the quality of milk powder largely determines its sensory attributes. Rates of enzymatic

and oxidative reactions were higher in those milks processed from older powders, and the taste of reconstituted UHT milk was more affected by lipolysis than by proteolysis. Besides, an astringent flavor described as powdery or chalky, and bitter was detected towards the end of storage time whereas lipolytic rancidity could be perceived earlier during storage (Celestino *et al*., 1997b).

15.3.4.2 Milk powder, sterilized, and concentrated milk

The sensory quality of milk powder is greatly affected by the initial milk quality, processing variables, storage conditions, packaging type, presence of physical and chemical agents (oxygen, light, water activity, antioxidants), and the extent of post-processing contamination. Flavor and the volatile profile of dried milks can also undergo seasonal variation and change during storage. A study revealed that WMP manufactured in summer had significantly higher levels of hexanal, pentanal, and dimethyl sulfide than that obtained in autumn and winter. Butanoic acid showed also significant differences between autumn and spring (Biolatto *et al*., 2007).

During storage an overall decrease in milk powder quality is evidence of a direct consequence of off-flavor development. Flavor variability in milk powder may negatively impact consumer acceptability when it is used in reconstituted milk or in ingredient applications (Caudle *et al*., 2005).

Owing to the low water activity that characterizes milk powder, it is accepted that microbial growth does not take place (Chen *et al*., 2003). However, heat-stable enzymes from spoilage bacteria in raw milk retain their activity after thermal processes such as evaporation and spray drying, consequently reducing its shelf-life. Lipolysis can occur in dried products in spite of the low moisture level. Powder manufactured from raw milk kept refrigerated for 48 hours had a higher lipolysis than the product made with fresh raw milk (Celestino *et al*., 1997a). Levels of short-chain fatty acids above the threshold values were also reported in WMP after storage for 2 weeks at 37°C (Chen *et al*., 2003). Astringency and bitterness caused by action of proteases on β-casein (Harwalkar, 1972) or by interaction between whey proteins, calcium phosphate, and caseins (Karagül-Yüceer *et al*., 2002) is a defect that can also be detected in high-heat-treated milk such as sterilized milk, concentrated milk and dry milk products (Lemieux & Simard, 1994; Karagül-Yüceer *et al*., 2002).

Lipid oxidation and non-enzymatic browning are the main sources of off-flavor in dried and sterilized concentrated products (Chen *et al*., 2003; Farkye, 2006), since lipid oxidation is the main factor limiting the shelf-life of WMP. Grassy, painty, staleness, and oxidized are typical off-flavors reported in WMP linked to lipid oxidation. During storage of WMP it was observed that as concentrations of certain compounds such as dimethyl sulfide, branched-chain aldehydes, straight-chain aldehydes, 1-octen-3-ol and 3-octen-2-one increased, grassy and painty flavors increased but other flavors such as cooked, sweet aromatic, and milk fat flavor decreased (Lloyd *et al*., 2009b). Taking into account that milk fat has a key role in the development of flavor defects, the kinetics of the formation of volatile fat oxidation products and other volatile compounds in WMP has been investigated (Hall *et al*., 1985). The heat treatment of milk prior to the manufacture of milk powder is the major factor controlling the oxidative damage. High temperatures of preheat treatments increase the antioxidative capacity but the cooked flavor is intensified (Stapelfeldt *et al*., 1997; Lloyd *et al*., 2009b). Packaging materials must be carefully chosen protecting the powder from moisture, oxygen, light, contamination, and microorganisms (Farkye, 2006). It has been documented that the presence of air in WMP samples packaged in plastic laminate pouches promotes the development of grassy and painty notes whereas nitrogen flushing extends the shelf-life. Therefore, packaging headspace oxygen should be maintained as low as possible to prevent off-flavor (Lloyd *et al*., 2009a). Storage at temperatures above room temperature accelerates autoxidation processes (Stapelfeldt *et al*., 1997); in this sense, refrigerated storage has been proposed to prolong the stability of WMP by delaying oxidative deterioration (Lloyd *et al*., 2009a). The incidence of water activity on oxidative stability seems to be affected by storage temperature and moisture level (Stapelfeldt *et al*., 1997; Farkye, 2006).

Carbonyl compounds and mainly straight-chain aldehydes are considered useful markers to monitor flavor defects of WMP caused by lipid autoxidation (Ulberth & Roubicek, 1995). Hexanal level was reported as the best predictor of grassy flavor, and hexanal or nonanal concentrations were considered good predictors of painty flavor (Lloyd *et al*., 2009b). Some values of sensory thresholds of flavor defects in reconstituted WMP are available in the literature (Hough *et al*., 1992).

The Maillard reaction in dried milk products is much faster than in fluid milk due to the lowest water activity and the storage at room temperature (van Renterghem & De Block, 1996; van Boekel, 1998). Furosine formation was proposed as a marker for this reaction, as its level is highly affected by the drying process and storage conditions (van Renterghem & De Block, 1996). The Maillard reaction has been especially investigated as another source of compounds responsible for staleness in milk powder. Benzothiazole and *o*-aminoacetophenone were reported as the main contributors (Arnold *et al*., 1966; Karagül-Yüceer

et al., 2002). However, other authors have indicated that stale is a composite off-flavor, with at least 12 compounds (including alkylpyrazines, aromatic aldehydes and pyrroles) involved in this defect (Ferretti & Flanagan, 1972).

In addition to lipid oxidation and the Maillard reaction, other chemical reactions can generate the onset of undesirable notes in milk powder. One of the first atypical flavors identified in dry milk was the result of chemical reactions involving ozone in the dryer air; 6-*trans*-nonenal is the major contributor to this defect (Parks *et al*., 1969). More recently, compounds thermally generated such as pyrrolines, thiazolines, and thiazoles have been suggested as possibly responsible for cereal-type off-flavor (Karagül-Yüceer *et al*., 2002). Methional has also been reported as an off-flavor compound in milk powder, whose aroma was described as boiled potato-like. In this product, methional can come either from Strecker degradation or light action on methionine (Karagül-Yüceer *et al*., 2001, 2002). Other compounds such as *p*-cresol and skatole may be contributors of animal-like, cowy, or fecal off-flavors, and their origin seems to be related to cow feeds (Karagül-Yüceer *et al*., 2002). Similarly, an undesirable odor described as cowbarn-like was reported, but its cause could not be elucidated. Tetradecanal, β-ionone, and benzothiazole were the compounds more closely related to this defect (Shiratsuchi *et al*., 1994b). Fortification of non-fat milk powder with vitamin A may often generate a typical hay-like off-flavor, which has been attributed to products formed by thermal oxidation of vitamin A palmitate (Suyama *et al*., 1983).

15.3.4.3 Infant formula

In infant formulae, sterilization or dehydration is absolutely necessary to provide an adequate bacteriological safety and to prolong their shelf-life (Albalá-Hurtado *et al*., 1998; O'Callaghan *et al*., 2011). However, these processes can affect the sensory characteristics. Similarly to milk powder, the main defects are led by Maillard reactions and fat oxidation. Infant formulae combine a number of factors that favor non-enzymatic browning: high quantities of lactose and lysine, relatively high temperatures, and a long time of storage (Ferrer *et al*., 2000b; Chávez-Servín *et al*., 2005). The formation of furfural compounds at advanced stages of Maillard reactions is considered a useful parameter to evaluate the degree of non-enzymatic reactions in infant formulae (Ferrer *et al*., 2000b). Numerous studies have shown a significant increase in furfural levels in infant milks during different conditions of storage (Albalá-Hurtado *et al*., 1998; Ferrer *et al*., 2000b, 2002, 2005; Chávez-Servín *et al*., 2005). On the other hand, the end of shelf-life in powder infant formulae is also affected by fat oxidation. Straight-chain aldehydes originating from unsaturated fatty acid degradation such as propanal, pentanal, and hexanal were proposed as potential indicators of infant milk powder oxidation (Romeu-Nadal *et al*., 2004). Hexanal content was found to vary from roughly 500 to 3500 μg/kg for non-oxidized and oxidized infant formulae, respectively (Fenaille *et al*., 2003).

15.3.5 Volatile profile and sensory characteristics of non-thermally treated milk

Although thermal treatments are considered the more effective methods to destroy microorganisms, some disadvantages related to chemical modifications of milk constituents or changes in the flavor have been claimed. Non-thermal processing technologies such as microfiltration, ultrasonication, pulsed electric field, high pressure, and microwave have emerged as new alternatives that allow inactivation of spoilage and pathogenic microorganisms while maintaining chemical properties of milk. Consumer perception about non-thermal treatments is that they provide more natural or fresher foods than heat treatments. However, at this time, the physical processes employed to reduce microbial loads in milk are unable to reduce the spore counts sufficiently to produce a safe product. As a consequence, they are often applied in combination with heat treatments (Deeth & Datta, 2011a). So far, few studies have been conducted to assess the effect of non-thermal processes on the volatile profile and/or sensory quality of milk. The assayed technologies have been microfiltration (Elwell & Barbano, 2006; Rysstad & Kolstad, 2006), ultrasound (Riener *et al*., 2009; Chouliara *et al*., 2010; Engin & Karagül-Yüceer, 2012), pulsed electric fields (Zhang *et al*., 2011), microwave (Valero *et al*., 1999, 2000; Clare *et al*., 2005), high hydrostatic pressure processing (Vazquez-Landaverde *et al*., 2006b), and ultra-high-pressure homogenization (UHPH) (Pereda *et al*., 2008a).

15.3.5.1 Microfiltration

The development of membrane technology has revolutionized the field of dairy processing (Pouliot, 2008). Microorganisms are removed according to their bacterial size, unlike pasteurization which is designed to destroy any microbiological danger in the food (Rysstad & Kolstad, 2006). Microfiltration has proved its capacity to eliminate bacterial cells and spores as well as to increase shelf-life when it is combined with mild pasteurization (Avalli *et al*., 2004; Deeth & Datta, 2011a). Pretreatment by cross-flow microfiltration of milk with a cell load reduction up to 4 log cfu/mL is used for the production of low heated fluid milks having a flavor similar to that of raw milk and a shelf-life three to five times longer than that of classical products (Saboya & Maubois, 2000; Elwell & Barbano, 2006). However, since the enzymes are

not inactivated by microfiltration, changes in flavor by proteolysis and lipolysis during storage could be expected. This is dependent on initial somatic cell count of raw milk and storage temperature (Elwell & Barbano, 2006).

15.3.5.2 Ultrasound

Ultrasonic treatment has attracted considerable interest in food science and technology. This emerging technology is based on the use of sound waves above the frequency of human hearing (>18 kHz). Over the last decade, an increase in potential applications of ultrasound in the field of food processing and preservation has been observed (Knorr *et al*., 2004; Dolatowski *et al*., 2007). The effects of ultrasonic waves on physicochemical characteristics, sensory properties, shelf-life, enzymes, and microorganisms of milk as well as application in the dairy industry for the homogenization process have been reported (Bermúdez-Aguirre *et al*., 2009; Cameron *et al*., 2009; Ashokkumar *et al*., 2010; Chouliara *et al*., 2010; Engin & Karagül-Yüceer, 2012). Although ultrasonicated milks seem to offer an alternative to pasteurization, many aspects of ultrasound mechanisms such as cavitation phenomena (formation and violent implosion of bubbles) and heating (conversion of acoustic energy to heat) (Villamiel & de Jong, 2000; Deeth & Datta, 2011b) have not been fully elucidated. In addition, milk flavor can be affected by certain chemical reactions that may occur during the sonication process such as oxidation by formation of hydroxyl radicals and hydrogen atoms or pyrolysis (Makino *et al*., 1983). In particular, the generation of volatile compounds in milk treated by ultrasound (Riener *et al*., 2009; Chouliara *et al*., 2010; Engin & Karagül-Yüceer, 2012) has been investigated. Some compounds derived from lipid oxidation such as aldehydes and hydrocarbons appear to be related to this treatment. An undesirable rubbery aroma has been detected in sonicated milks but the responsible components were not yet identified (Riener *et al*., 2009).

15.3.5.3 Pulsed electric field

Pulsed-energy or pulsed electric field has gained increasing attention as an alternative to traditional food processing. This technology utilizes very short electric pulses (nanoseconds to milliseconds) at high electric field intensities at moderate temperatures (Bendicho *et al*., 2002). Electric field strength and treatment time are two of the most important factors involved in pulsed electric field processing (Jeyamkondan *et al*., 1999; Bendicho *et al*., 2002). In the last decade, the effectiveness of different processing parameters on microbial and enzyme inactivation and functional properties of milk has been evaluated (Bendicho *et al*., 2002; Floury *et al*., 2006; Noci *et al*., 2009; Bermúdez-Aguirre *et al*., 2011). In spite of some controversial results, pulsed electric field is considered a promising technology to replace, at least partially, the traditional thermal treatments of liquid foods or to extend the shelf-life of pasteurized milk (Sepulveda *et al*., 2005; Deeth & Datta, 2011b). Few studies have considered the effect of pulsed electric field treatment on volatile fraction. Sampedro *et al*. (2009) observed that pulsed electric field treatment applied to orange juice and milk-based beverages achieved the same degree of microbial and enzyme inactivation as thermal treatment, but better preserved color and volatile profile. Zhang *et al*. (2011) found some differences in the volatile fraction of milks subject to pulsed electric field compared with pasteurization, but these differences were not detected by olfactometric analysis. Little or no change in the sensory properties of milk was also reported (Bendicho *et al*., 2002; Sepulveda *et al*., 2005; Deeth & Datta, 2011b). Therefore, it has been claimed that pulsed electric field processing maintains the freshness of foods (Bendicho *et al*., 2002).

15.3.5.4 Microwave

Microwave treatment has been suggested as a reliable method of heating since the milk is not exposed to overheated exchange surfaces (Clare *et al*., 2005). Results obtained from the comparison of volatile compounds between microwave-heated milks and conventionally heated milks have demonstrated that microwave technology is a useful alternative for milk processing since the sensory characteristics of this milk are equivalent to, if not better than, those exhibited by conventional processing (Valero *et al*., 1999, 2000; Clare *et al*., 2005).

15.3.5.5 High hydrostatic pressure

High hydrostatic pressure or high-pressure processing is being looked at with particular interest because the nutritional and sensory properties of food seem to be not affected (Mussa & Ramaswamy, 1997). Although it has been recognized as an alternative to thermal processing, the resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and treatment duration and type of microorganisms (Trujillo *et al*., 2002; Fonberg-Broczek *et al*., 2005). The application is generally performed at pressures ranging from 300 to 600 MPa for 2–30 min (Deeth & Datta, 2011c) and at ambient temperature. Like other technologies, the effect of high pressure on microorganism destruction, and physicochemical and sensory properties of milk have been investigated (Pandey *et al*., 2003; Fonberg-Broczek *et al*., 2005; Altuner *et al*., 2006; Huppertz *et al*., 2006). An interesting review about applications of high hydrostatic pressure on milk and dairy products by Trujillo *et al*. (2002) is available. Information about the impact of high

hydrostatic pressure on the volatile fraction of milk and the mechanisms of volatile generation is still very scarce. A small effect on covalent bonds, smaller organic molecules such as volatile compounds and vitamins was reported (Trujillo *et al*., 2002). However, other studies have indicated a strong effect of high hydrostatic pressure on compounds produced from the Maillard reaction (Frank *et al*., 2002). In fact, the effect of high hydrostatic pressure on the volatile profile seems to be highly dependent on the processing temperature. Vazquez-Landaverde *et al*. (2006b) observed that high-pressure processing in the range 480–620 MPa at low temperature (25°C) has a minor effect on volatile components of milk, and particularly methyl ketones and aldehydes were not formed at any applied pressure, suggesting clear advantages compared with thermal treatments. However, a combination of high pressure and high temperature (60–80°C) increased dramatically the concentration of aldehydes. It has been hypothesized that oxygen is more soluble under high pressure and hence it could promote hydroperoxide formation and consequently increase aldehyde production.

15.3.5.6 Ultra-high-pressure homogenization

The most recent development is a new high-pressure process called ultra-high-pressure homogenization (UHPH), which is based on the same principle as conventional homogenization but works at higher pressures (up to 400 MPa) (Pereda *et al*., 2009). Results from microbial inactivation, physicochemical parameters and shelf-life from milk subject to UHPH processes indicate its suitability to replace the conventional thermal treatments (Pereda *et al*., 2007, 2008b, 2009; Pedras *et al*., 2012). Comparison of volatile profiles between milk samples subjected to thermal processing (pasteurization, UHT and sterilization) and different UHPH conditions revealed that whereas heat treatments produced an increase in aldehyde and methyl ketone contents as thermal intensity increased, UHPH technology induced an increase in aldehydes alone, which was more pronounced as the value of the applied pressure increased (Pereda *et al*., 2008a).

15.4 MILK FROM MONOGASTRIC SPECIES

Monogastric species, such as humans and horses, produce milks with nutritional, sensory, and physicochemical characteristics that differ from the milk of ruminant species, while the camel is considered a pseudo-ruminant with a stomach anatomy similar to, but modified from, that of true ruminants. Human and camel milks have been the most studied, because of their relative importance compared to other species. An example of differences in composition to bovine milk is the fact that β-casein is the major protein in camel and human milk, and they do not contain β-lactoglobulin (El-Agamy, 2006; Morgan, 2006; Konuspayeva *et al*., 2009; Al-haj & Al-Kanhal, 2010).

Sensory properties of camel milk include an opaque and white color and acceptable taste, which is normally described as sweet, sharp, and sometimes salty. The taste changes according to the type of plant consumed by the camels and availability of water (Al-haj & Al-Kanhal, 2010). In turn, sensory evaluation of fresh human milk has revealed very low odor intensity (practically odorless), and its attributes were described as predominantly sweet, fatty and soybean-like, with slight buttery and cooked milk-like notes (Spitzer & Buettner, 2010).

The volatile profile of human milk is the result of the delicate balance of several compounds of different chemical classes such as aldehydes, ketones, fatty acids, and terpenes (Sandgruber *et al*., 2011). Analysis of human milk samples carried out by Shimoda *et al* (2000) has shown a volatile profile quite different from that of cows' milk. Aldehydes were the most important group of compounds in human milk among which unsaturated and long-chain carbonyl compounds were the most representative of the volatile fraction. In addition to these compounds, lactones and fatty acids are commonly found (Spitzer & Buettner, 2010). The main difference in flavor perception between human and bovine milk seems to be sweetness, which is higher in human milk (McDaniel *et al*., 1989). When samples of both types of milks are stored at –19°C, significant differences in the intensities of other attributes such as hay and fishy flavor have been observed (Spitzer & Buettner, 2010).

Today, breastfeeding is often performed by pumping the mother's milk and storing it until use, which can result in flavor changes or in development of harming constituents. Human milk seems to be a food matrix highly sensitive to sensory deterioration, being mainly affected by oxidative and lipolytic processes that involve free fatty acids (Sandgruber *et al*., 2012). In this regard, the analysis of several storage conditions on the stability of the triacylglyceride fraction of human milk indicated that storage only at –20°C without previous heat treatments led to the appearance of free fatty acids (Morera Ponds *et al*., 1998). Recently, several studies have been conducted on the influence of different storage conditions on human milk flavor. Spitzer and Buettner (2010) evaluated the sensory changes in human milk samples after 2 months' storage at –19°C. They found that the aroma profile of breast milk can be considerably affected by storage at the recommended conditions. Several volatile compounds such as 1-octen-3-one, (Z)-1,5-octadien-3-one, *t*-4,5-epoxi-(E)-z2-decenal, (E,Z,Z)-2,4,7-tridecatrienal, (E,Z)-2,4-nonadienal, and (E,Z)-2,4-decadienal were significantly increased in stored samples. Some of these carbonyl compounds could be responsible for the intense and characteristic fish-like and

metallic off-flavors that develop in this type of milk. In addition, rancid, soapy, and sweaty notes were detected, which were attributed to increased concentration of free fatty acids. In a subsequent work, Spitzer *et al*. (2010) reported that the heating of milk prior to freeze storage induced slight flavor changes, with generation of an egg-white-like note but with no further generation of off-flavor during storage. Sandgruber *et al*. (2012) observed a delay in off-flavor development by oxidative processes if storage of human milk is performed at –80°C.

Taking into account the important variations observed in specific odorants between milks of different mothers, maternal diet has been proposed as another possible source of flavor changes in milk, mainly due to transmitted compounds. A recent study investigated whether specific fish oil odor constituents can be transferred to mothers' milk (Sandgruber *et al*., 2011). Surprisingly, data obtained from sensory profiles and chemical markers showed that no statistical differences occurred between milks from mothers after long-term fish oil supplementation and the control group. Today, this topic is being considered.

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