Milk Mixtures

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This entry describes the most important research on cheeses obtained from processing mixtures of different milk species and discusses the effect of milk species and their mixture ratios on cheeses features (biochemical composition, physicochemical and rheological characteristics, sensory properties, and microbial ecosystem).

Keywords: cow ; ewe ; goat ; camel ; buffalo ; milk mixtures ; cheese ; physicochemical properties ; sensory features ; microbial ecosystems

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1. Introduction

Milk and dairy products are considered as a potential resource for providing functional foods [1]. This is related to their content of a variety of essential components such as proteins, polyunsaturated fatty acids (FAs), vitamins, minerals, and also to the simplicity of incorporating lactic acid producing bacteria (LAB) during their manufacture. During the last few decades, camel milk (CaM), goat milk (GM), ewe milk (EM), and buffalo milk (BM) have received special attention. This is principally due to their recognition as exhibiting a higher potential for functional foods from a nutritional point of view when compared to cow milk (CM).

Cheese represents one of the most popular food products in the world. This is probably thanks to its richness in nutritional components like proteins, short-chain FAs, vitamins (e.g., riboflavin, thiamin, vitamin B12), and minerals (e.g., calcium, phosphorus) [2]. The number of scientific studies conducted on the characterization of cheeses produced from mixtures of different species has increased from year to year. The production of 150 papers was observed from 1990 to 2008, while 350 papers were published from 2014 to 2019 (Scopus database on 1 July 2020; keywords: cheese, mixture and milk).

2. Sensory and Rheology Features of Cheese

Mallatou et al. [3] reported what are probably the first results concerning the effect of mixing milk from different species on the sensory properties of cheese. During the processing of Feta cheese, they noted that pure EM cheese had the highest scores in terms of body-texture than the other cheeses produced by mixing EM and GM. Statistical analysis showed that the force necessary to cause fracture in cheese samples made from pure GM was higher than the samples made from 50% GM (1GM:1EM), 25% GM (1GM:3EM), and 100% EM. This suggested that the softest cheeses were found to be pure EM and the hardest ones were those of pure GM. Freitas et al. [4] reported contradictory results. Indeed, they claimed that the volumetric ratio of EM to GM milk was not statistically significant in terms of its effect upon surface, form, and texture of cheeses. However, contrary to Mallatou et al. [3], they observed that cheeses manufactured with either 75% of GM (1EM:3GM, v/v) or pure GM received the best scores for texture. Regarding Minas fresh cheese processes from CM and GM, Sant'Ana and coworkers [5] also noted that the 1CM:1GM (v/v) cheese displayed a difference only at the beginning of storage (day 1) when compared with the other cheeses (pure CM and pure GM). When the analysis of the texture profile (TPA) was investigated, the authors noted that the type of milk used to manufacture cheese and the length of storage did not affect the adhesiveness, elasticity, or cohesiveness of the cheeses. Nonetheless, the CM and 1CM:1GM (v/v) cheeses displayed decreased gumminess and chewiness values over the storage period evaluated, whereas this behavior was not observed in GM cheeses.

Niro et al. [6] reported the comparison between the effects of adding EM or GM into CM milk on cheese texture properties. During their investigation, they managed three pasta filata cheese productions made with 4.55CM:1EM (v/v), 1.86CM:1GM (v/v), and pure CM and noted higher scores for elasticity and adhesiveness of the pure CM cheeses. Moreover, higher friability was observed for the cheeses containing EM, corroborating the investigations of Mallatou et al. [3] and Tsigkros et al. [7].

In a different approach, Shahein et al. [8] explored the potential for mixing CaM and BM on the body/texture of Domiati cheese. The authors studied five ratios (9CaM:1BM, 4CaM:1BM, 2.33CaM:1BM, and 1.5CaM:1BM, v/v) and their respective pure milks (pure CaM and pure BM). As noted for mixtures containing CM, the authors observed that adding BM improved the body/texture of cheeses after 45 days of pickling. These results are in agreement with those reported by Shahein et al. [8].

The differences in the cheese texture parameters observed by the aforementioned studies can be attributable to different factors: the initial composition of milk and cheese (fat, protein and moisture), the manufacturing process (brining, dry salting), and the extent of the proteolysis index [9]. For example, it has been shown that high acidity, protein, and total solids content generally make cheese harder and less easily deformed [10][11]. Conversely, a high-moisture-content of cheese is associated with a fragile protein network that results in less-firm cheeses [12] and a higher degree of unsaturation of FAs is correlated with a smoother texture [13]. Despite these different factors and regardless of dairy species (i.e., cow, goat, and sheep), casein gels are responsible for most of the various rheological/textural properties of cheese, stretch and fracture [14]. It has been shown that smaller micelles form a more compact and, hence, firmer gel network than larger micelles [15][16], whereas contradictory results have been obtained for rennet clotting time [17][18]. Concerning the differences observed between the sample containing EM and CM, these disparities might be due to different casein structures or concentrations in the milk, where ovine milk contains higher levels of casein than caprine milk [19]. GM differs from CM in several physicochemical characteristics, which explains major differences in the technological behavior of the two types of milk [20]. The poorer cheese making ability of GM is largely attributable to the lower casein content, and to specific properties of casein micelles in GM such as their composition, size, and hydration [20]. GM also has different proportions of the four major caseins compared to its CM counter-parts, and there are great variations, especially between αs1-casein and αs2-casein content between individuals and breeds of goats and sheep due to the occurrence of genetic polymorphisms of all milk proteins, which greatly influence their cheese making properties [20].

Concerning flavor properties, different authors have investigated the effect of mixing milk from different species on this important cheese feature. From this perspective, Queiroga et al. [21] studied the effect on the sensory features of Coahlo cheeses obtained after mixing CM and GM. The authors reported that increasing the proportion of the cheese milk to 50%, bovine had no significant effect on the fruity flavors that are associated with CM cheeses. However, waxy/goaty and bitter flavors were present at lower intensities than in cheeses manufactured from 100% GM. Sheehan et al. [22], on semi-hard cheeses, confirmed these previous investigations and associated the decrease in goaty flavor mainly to a reduction of flavor compounds such as methyl and ethyl esters of short chain acids and terpene. Sant'Ana et al. [5] also highlighted in Minas fresh cheese that the higher short-chain FA contents promote the typical aroma and flavor of the dairy products made with GM [23]. Those conclusions were reported after having compared cheeses formulated with 1CM:1GM (v/v) and cheeses containing only CM or GM. Nonetheless, they observed that the three categories of cheeses did not differ in their salty and acidic flavor. The CM cheese was described as exhibiting a better wet appearance compared with cheeses containing GM (pure GM and 1CM:1GM (v/v) cheeses). This difference in the wet appearance corroborated the higher syneresis observed by the authors during CM processing. CM cheese received higher scores for butter flavor and aroma, whereas GM cheeses received higher scores for global aroma and flavor when compared with CM and CM:GM cheeses. These results matched those of Niro et al. [6]. During their investigations, they noted higher scores regarding sweet attributes for pasta filata manufactured from pure CM compared to cheeses produced from mixing CM and EM or CM and GM. In the same study, Niro and coworkers claimed that samples containing CM and EM (4.55CM:1EM) displayed higher scores for intensity of flavor, acidic, astringent, and salty attributes after 30 days of ripening. In contrast, samples containing a mixture of GM and CM were found to exhibit high solubility, together with the intensity of aroma and bitter attributes. Moreover, CM cheeses presented higher scores regarding sweet attributes when compared to other cheeses.

The conclusions above-mentioned are in contradiction with the investigations of Freitas et al. [24]. Indeed, the researchers reported, after the analysis of 1GM:1EM, 1GM:3EM, and 3GM:1EM (v/v) cheeses, that the volumetric ratio of EM to GM milk was not statistically significant in terms of its effect upon flavor and overall sensory parameters of cheeses. However, contrary to Mallatou et al. [3], they observed that cheeses manufactured with either 75% GM (1EM:3GM, v/v) or pure GM, and ripened for 180 days received the best scores for flavor. Moreover, they observed that cheeses made from pure GM exhibited the second highest score, while the other cheeses had no significant differences regarding flavor. Even when cheeses were made from pure GM, no panelists detected a goaty flavor, contrary to the study of Queiroga et al. [21]. However, no significant difference between pure GM and cheeses containing 75% GM (3GM:1EM) was reported. As the storage period was prolonged from 60 to 120 days, the authors noted that the sensory quality of Feta cheeses produced from GM deteriorated, and, in contrast, that made with EM improved.

When considering camelids, Siddig et al. [25] reported that mixing CaM with CM (i.e., 1CaM:1CM) to produce Jibna-beida cheese did not decrease the acceptability of the final products. All of the cheeses were highly rated by the panelists with preference to cheese prepared by using starter culture, which was better received compared to that prepared by an acidification process. It can be concluded that production of an acceptable quality Jibna-beida cheese from a mixture of CaM and CM is feasible.

Concerning color, most of the studies reported the effect of mixing different milk species on the whiteness, yellowness, and redness of cheeses after analysis by a sensory panel or physical measurements (e.g., chromameter). In this context, Queiroga et al. [21] depicted that Coahlo cheeses made from 1CM:1GM (v/v/) and pure GM presented higher L* (white component) values from seven days of storage onward. These results were in contradiction with Sheehan et al. [22], who reported no effect of milk type on cheese whiteness. Nonetheless, cheeses made from GM are generally whiter in color [26]. This is related to the fact that goats can convert β-carotene into vitamin A and also produce milk with smaller-diameter fat globules compared to that produced by cows [27][28]. Moreover, Álvarez et al. [29] observed a positive correlation between the moisture content and L* in cheeses made with GM, suggesting that a high moisture content results in lighter products. Higher a* values (red component) were found in GM cheeses. According to Sheehan et al. [22] the increase in a* value in cheeses is directly related to the addition of GM and more specifically to their FA profiles. The b* values (yellow component) were found to be higher for CM cheeses. This difference in color is assigned to a high-level transfer in cows of carotenoids from their diet to milk and, consequently, dairy products made with CM are more yellow than those prepared with milk from other species [30]. These results corroborated Sant'Ana et al. [5] and Ramírez-López and Vélez-Ruiz [31] on Minas fresh cheese (GM, CM and an equal mixture of both) and Fresh Panela cheeses (1GM:9CM, 1GM:4CM, 1GM:2.5CM, 1GM:1.5CM, v/v), respectively.

For cheeses processed from CaM, Shahein et al. [8] noted no difference in appearance/color on Domiati cheeses (soft pickled cheese) made from different mixtures of CaM and BM (9CaM:1BM, 4CaM:1BM, 2.33CaM:1BM, and 1.5CaM:1BM, v/v) and their respective pure milks (pure CaM and pure BM) during storage. These results are in agreement with that reported by Shahein et al. [8].

3. Microbial Ecosystems of Cheese

Milk microbial ecosystems are the main agents that contribute, via their metabolic activity, to the quality of dairy products in terms of flavors, aroma, and texture, but also with regard to safety. Numerous intrinsic and extrinsic factors drive the composition and richness of these communities, among them farm practices of dairy herd management, feed quality, season, stage of lactation, animal health, weather conditions, water quality, and hygienic practices of the milking stage are the most frequently described [32]. In this sense, so-called special composition milks are particularly relevant models. More than bacteria present in milk at a given time, what matters is the expression of their metabolic functions together with their interactions. Indeed, structure and organization of the bacterial community is determined by the chemical composition of milk and, more precisely, the nutritional content and accessibility, and the presence or not of immunological factors and other antimicrobial agents.

All the studies converge to show that the microbial loads and composition of raw milk before any treatment vary considerably between geographically distant farms, among herds from the same region and even among samples from the same animal or herd. Accordingly, considering these variations, the comparison of published data about the microbial composition of milk from different species all over the world can therefore be misleading. Moreover, from culture-based enumeration and phenotypic identification methods to high-throughput sequencing approaches, the information gathered also differs. The first aims at the quantification of taxonomic or functional microbial “groups” that are able to grow on rich and/or selective media. The second, which is based on culture-independent DNA-based technologies detects dead cells as well as extracellular DNA and viable populations. As explained by Metzger et al. [33], in milk, bacterial DNA originates mainly from the animal's skin or environment, from the keratin of the teat canal, from leukocytes in the milk, and milk within the mammary gland environment. Accordingly, one can assume that at the tank level, the presence and diversity of bacterial populations relies mainly on the way that the milking process favors or limits the transfer of bacteria to raw milk. Nevertheless, several authors have managed the description of milk microbiota from different occidental animals (cow, goat, sheep, buffalo) [34] and identified that most of the bacteria belongs to four main phyla: *Proteobacteria, Actinobacteria, Firmicutes*, and *Bacteroidetes*. At the genus level, *Pseudomonas* spp. are often predominant but its environmental origin suggests a link with milking hygiene more than the animal itself. LAB such as *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, and *Weissella* are omnipresent, but their proportions vary greatly. According to the sample origin, a great diversity of other bacteria detected in low abundance is often observed.

Considering mixtures of milk from different animal species, it is likely that at the time of mixing, total microbial composition would be the simple addition of microorganisms from each milk. However, a few minutes later and during the subsequent transformations from milk to cheese or other dairy products, the questioning is different. The multiple and dynamic variations of biotic and abiotic factors would determine the metabolic activity of bacteria, yeast, and molds as well as their interactions. Indeed, the different strains might have unequal levels of adaptability after contact with the components of their non-native milk varieties or changes during the cheese-production process. All around the world, numerous traditional cheeses or dairy products are manufactured from ruminant-milk mixtures. In most cases, these practices aim to overcome the low-volume of production of each herd and are the fact of small or very small dairy farms with poor hygienic practices. These studies put more emphasis on the spoilage and pathogen microflora (i.e., *Pseudomonas* spp., *Bacillus* spp., *Clostridium* spp., *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* 0157:H7, and *Staphylococcus*) and the way to control them including the diversity and antimicrobial properties of their LAB population (for review, see Alexandraki et al. [35]). Nowadays, industrialists from the dairy sector seek to develop new products exploiting the nutritional qualities of non-cow milks with the double aim to promote local resources and to fit the consumer demand. These products may include drinking milk, yoghurt, butter, and different types of cheese.

In 2014, Niro and collaborators presented innovative pasta filata cheeses made from a mixture of raw CM with EM (4.55CM:1EM) or GM (1.86CM:1GM). The process was similar to that of Caciocavallo, which consists of the addition of mesophilic and thermophilic LAB, followed by the addition of rennet in milk. In fact, the particularity of this cheese lies in the fact that curd formation and kneading are performed in hot water (80 °C) before salting and ripening for 2 months at 10 °C. Mixed CM:EM and mixed CM:GM cheeses presented higher mesophilic rod and cocci LAB counts in comparison with CM cheeses during the 60 days they were tested, but the latter exhibited a higher presence of thermophilic bacteria over the same period; it should be noted that the differences never exceeded 0.5 log. In all cheeses, the fecal coliforms were not detectable at 60 days of ripening. Moreover, total coliforms, *enterobacteria*, and molds were undetectable or stayed at very low levels in all cheeses.

4. Conclusions

Based on the above studies, it has been fully demonstrated that the knowledge of how (i) the animal species from which the milk originates and (ii) the proportions of each species used in the mixture influences the quality of cheese, is necessary to design products with improved physicochemical, nutritional, functional, and sensory qualities. Even though extensive research has been conducted on milk blends and their use in cheese production, many other characteristics of these products need to be investigated. For instance, it is evident that much attention has to be devoted to the microstructure, microbiology, and release of bioactive compounds in cheese obtained from milk mixtures. These three factors are of the utmost importance and require an appropriate understanding of their relation to the quality of cheese and dairy products in order to design bespoke products.

Moreover, concerning microorganisms, as far as we know, the microbiota of CaM has not yet been sequenced and conventional agar plate enumeration did not show the existence of a specific species, so there is a gap to fill. However, numerous studies have reported the presence of Lactobacillus species able to tolerate the condition of the gastrointestinal tract, while exhibiting antibacterial activity against bacterial pathogens. These strains could be good candidates as starters or probiotics [36]. Moreover, most research around non-cow milk has focused on the characterization of a few species that could present specific characteristics such as probiotic value or starter qualities [37][38]. It will be able to rely on the potentialities of metatranscriptomics and metabolomics techniques to understand the interactions between the microorganisms and the components of the dairy matrix because the release of bioactive compounds is closely related to the composition of dairy products.

Concerning bioactive compounds, it has been reported in the present review paper that some studies have focused their attention on peptides and FAs. Nonetheless, several compounds, like GABA, conjugated linoleic acid (CLA), vitamins, and exo-polysaccharides can be found in dairy products. Those compounds play an important role in both the development of the texture and in the flavor of cheese. At the same time, they can exhibit interesting antioxidant, antimicrobial, immunological properties, and show potential in disease prevention (e.g., obesity, dyslipidemia, and type 2 diabetes). These compounds can be naturally present in milk while others can be released by microorganisms during processing. For example, microorganisms like LAB can release several molecules in milk during cheese ripening (e.g., vitamins, exo-polysaccharides). Therefore, the identification of those bioactive compounds is of primary importance, as is their characterization via metabolomics, separation, and detection techniques.

Finally, we assumed that the development of appropriate technologies for the production of innovative cheeses using a mixture of cow, ewe, and goat milks among others, with proper characteristics and satisfactory acceptance by consumers, could be an interesting and feasible opportunity for the dairy industry, allowing its expansion in the market. It is also evident that special attention should be paid to applied and innovative research to adapt to the different non-cow milk sectors to future production and market trends.

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