

Characterization of cheeses produced with ovine and caprine milk and microbiological evaluation of processing areas in the dairy plant in Brazil

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Abstract

In this work, Fascal, Serra da Estrela-type, Roquefort-type, and Feta-type cheeses were produced with ovine milk and a Feta-like cheese was prepared with goat milk. The microbiological and physicochemical characteristics of these cheeses were determined and microbiological counts in milk, water and surfaces at processing areas were evaluated. Counts of total and fecal coliforms were below 3.0 and 2.7 log MPN/g, respectively, and *Salmonella* was absent in 25 g for all cheese samples, in accordance to the limits established by Brazilian legislation. Counts of coagulase positive staphylococci were higher than regulatory limits (3.0 log CFU/g) in Fascal's curd and Feta-like cheese, but decreased during ripening of Fascal cheese. Samples of milk and water in the dairy plant presented results in accordance with the standards. Monitoring the microbiological counts at processing areas showed higher counts in the air of milk reception area and at the bulk milk tank surface. All samples showed high fat values, being classified as fatty cheeses according with current Brazilian regulations. Because the moisture values, Fascal and Serra da Estrela-type cheeses may be classified as low moisture or hard cheeses and Roquefort-type and Feta-type cheeses as semi-hard cheeses. These results may be helpful to define a standard to the products.

Keywords

Raw milk
regional cheese
cheese quality
microbiological quality

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Introduction

Production of typical dairy products from ewe and goat milk can afford a profitable alternative to cow milk products owing to their specific taste, texture and healthy properties (Park *et al.*, 2007; Raynal-Ljutovac *et al.*, 2008). Sheep and goat milks can be used for different purposes and become a very important mean for the subsistence economy in undeveloped geographic areas (Brito *et al.*, 2006; Suárez and Busetti, 2006).

Producing cheeses from small ruminant milk may be advantageous rather than competing with larger plants in the production of less expensive commodity cheeses made from bovine milk (Casper *et al.*, 1998; Scintu and Piredda, 2007). Sheep milk contains approximately a double concentration of fat and protein, and a higher energetic value than cow milk (Park *et al.*, 2007). In addition, it is a more suitable source for cheese production compared to cow milk since ovine milk is characterized by a lower colloidal stability (Raynal-Ljutovac *et al.*, 2008). Therefore, it is necessary to expand the value-chain of ovine dairy products in view of market demands and consumer preferences.

One major problem of cheesemaking with raw milk is the adventitious microflora present, coupled with the lack of pasteurization of milk and sterilization of manufacturing tools that will eventually permit inclusion of undesirable microorganisms, all of which will undergo different evolution patterns throughout ripening. Such population dynamics is partially responsible for several important differences between cheeses, and for unique characteristics of traditional cheeses (Freitas and Malcata, 2000; Coda *et al.*, 2006).

The demand for specialty cheeses has increased during the last decade in Brazil. This has been particularly beneficial to small producers of cheeses from ovine and goat milk. In this context, Fascal, a semihard cheese traditionally made using raw ewe's milk, commercial rennet and starter culture, was developed in the Southern region of Brazil (Nespolo *et al.*, 2009; Nespolo *et al.*, 2010). Sheep and goat milk cheeses produced in this region are commercialized in the regional market and the definition of a standard product and the accepted range of variability are necessary to diffuse its consumption. The main concern is to provide acceptable safety and standard levels of quality in these cheeses, according to the

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increasing attention by the Brazilian guidelines toward food hygiene and safety aspects. The aim of this study was to investigate the microbiological and physicochemical characteristics of ovine and caprine cheeses produced in a small dairy plant located at southern Brazil and to monitor the microbiological quality at dairy processing areas.

Materials and Methods

Cheese manufacturing plant

Cheeses were manufactured in a small commercial dairy plant, in Viamão city, 30°04'52" S and 51°01'24" W, south of Brazil, which manufactures mainly sheep's milk derivatives. This cheese plant was located in a dairy farm, with 117 hectares and 250 Lacaune breed sheep, with a monthly milk production around 5,000 l. The cheese production is mainly concentrated on ewe's cheeses, although other cheeses are manufactured with goat milk. Ovine cheeses evaluated in this research were Fascal, Feta-type, Roquefort-type and Serra da Estrela-type. The caprine cheese was produced with pasteurized goat's milk (72°C/15 s). Fascal is considered a typical Brazilian ovine cheese and the formulation was developed in this dairy plant (Nespolo *et al.*, 2009). The manufacturing procedures for the other cheeses were similar to those used in their traditional production.

Manufacture of Fascal cheese

Cheese was made at the commercial cheese plant according to standard Fascal cheese-making procedures utilizing raw ewe's milk (Nespolo *et al.*, 2009). The milk was set at 31 to 32°C for 30 min and separated in vats. Vats were previously cleaned and sanitized thoroughly to minimize contamination during manufacture of the cheese. Commercial starter culture (MA, Danisco), which comprised a blend of *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* strains, was added. Inoculation was according to the manufacturer's specifications. Following inoculation, rennet (BV bovine rennet) was added with equivalent clotting power. The mixture was incubated for 20 min at 31°C to allow clotting. Afterwards, perpendicular vertical cuts were made in the curd. The coagulum was cut into 1-cm cubes, followed by incubation for additional 40 min. The curd was then slightly compressed for 15-20 min, in order to aid in whey removal. At the moment the curd was put into the plastic molds, the forms were marked for later sampling. The molds were pressed at room temperature: 1 kg for 20 min; 2 kg for 120 min; 2.5 kg for 90 min. Cheeses were turned over three times

during the three hours. The process was finalized by brining the cheeses for 60 min, in a sodium chloride solution (20% w/w). During ripening for 90 days, the cheeses were stored in a ripening chamber at 10°C and 75% relative humidity.

Manufacture of Serra da Estrela-type cheese

Serra da Estrela cheese is a handcrafted cheese from the region of Serra da Estrela, Portugal, and its production is protected by denomination of origin (Barbosa, 1990). This cheese is manufactured with raw ovine milk of Bordaleira breed and coagulated via plant rennet with Cardo (*Cynara cardunculus* thistle). The Serra da Estrela-type cheese was produced with ovine milk from Lacaune sheep breed and a commercial starter culture (MA, Danisco), using the traditional method for manufacturing (Barbosa, 1990; Freitas and Malcata, 2000). The milk was curdled and the milk clotting was complete after 1 h. Once the cheese was drained, the diameter of the mould was reduced until the desired size. This work was done through very slow hand pressing for about two hours. The cheese was removed from the mould and rubbed with salt, after which a band of fine cloth was wound round and tied with a knot. On the following day, the cheese was placed in a refrigerated chamber, protected from draughts and kept in a damp atmosphere, at temperature from 6 to 8°C (Barbosa, 1990). The ripening period ranged between 80 and 90 days.

Manufacture of Roquefort-type cheese

Roquefort is a blue cheese from the south of France, according to the European protected designation of origin, produced with the raw milk of the Lacaune, Manech and Basco-Béarnaise breeds of sheep (Masui and Yamada, 1996). The Roquefort-type cheese was produced using the described technique (Roquefort Confederation, 2009), with milk from Lacaune ewes. The milk was heated at 28-34°C and curdling was performed using commercial starter culture (MA, Danisco), instead chymosin. The curds were cut into small blocks to drain the excess of whey, and the mold culture (*Penicillium roqueforti*, Chr. Hansen) was added. Cheese curds were placed into moulds in order to give its shape to the cheese. It was then salted and turned regularly for about a week and the ripening process was placed in a cave for 120 days.

Manufacture of Feta-type cheese

Feta cheese is a white and brined curd cheese, which is traditionally made in Greece and has been a protected designation of origin product in the

European Union. Traditionally, Feta cheese could be prepared by either thermized or raw ewe's milk by using only rennet and without the addition of any starter cultures (Kourkoutas *et al.*, 2006). For production of Feta-type cheese, ovine milk from Lacaune sheep was thermized (in open vat, at 63°C, for 30 min). The coagulation was performed with commercial starter culture (MA, Danisco) at 35-38°C, and cutting was done after 50 min. The filled molds were placed with a certain inclination, in order to enhance whey removing. After 3 h, cheese curd was cross cut, salted with salt of rice grain size and the molds with their lids were turned upside down. After 12-14 h, the Feta pieces were placed into plastic barrels in layers with dry salt between them. Feta pieces were taken out of the plastic barrels, washed carefully and placed in a brine solution. The ripening period was 60 d at 16-18°C.

Manufacture of goat cheese

The goat cheese was produced by the same procedure to that used for Feta-type cheese. Nowadays, most Feta cheese is produced from ewe's milk, goat's milk, or a mixture of both (Kourkoutas *et al.*, 2006). The goat milk was obtained from Saanen goat breed, not in the dairy farm, but it was from the same geographic region. This cheese was sampled during the ripening period, at 30 d of ripening.

Cheese, milk and water sampling

Cheese samples were collected after the production or during the ripening. Samples of raw ovine milk were collected from the refrigerated tank, before cheese production. Samples were collected in sterile plastic or glass specimen containers and kept under adequate refrigeration until analysis. Ovine milk and ewe's and goat's cheeses were analyzed on duplicate samples. Each sample was divided in parts for microbiological analyses and physical-chemical analyses. Water samples also were analyzed in duplicate. The results were compared to the levels permitted by the Brazilian legislation for cheeses (MAPA, 1996; ANVISA, 2001), milk (MAPA 2002) and water (ANVISA, 2004).

Microbiological analyses

After removed the rind, 10 g fractions from each cheese sample were obtained by cutting slices, each containing equal quantities of the innermost, intermediate and outermost parts. These 10 g sub-samples were aseptically handled and homogenized with 90 mL of sterile saline for 2 min in a blender. After filtering through sterile gauze, the homogenate was decimally diluted in sterile saline and selected

dilutions were plated in duplicate on the specific media required for the different microbial groups. For milk and water samples, 10 mL from each sample were collected after homogenization. The microbiological analyses were selected based on the Brazilian legislation for cheeses (ANVISA, 2001), milk (MAPA, 2002) and water (ANVISA, 2004).

Mesophilic aerobic bacteria were enumerated by spread plating on Plate Count Agar (PCA, Oxoid), incubated at 35°C for 48 h. The Presumptive and Confirmed Tests for coliform group were evaluated on 3-replicate, 3 dilution tube MPN procedure, at 35°C. The fecal coliform group was determined by EC Broth MPN Method at 45°C (APHA, 2002). Coagulase positive staphylococci were enumerated by spread plating on Baird Parker agar (Oxoid) at 35-37°C for 48 h, confirmed using coagulase test (APHA, 2002).

Detection of *Salmonella* spp. was carried out on 25 g samples, and the presence-absence determination was performed based on Salmonella Species Presumptive and Confirmation Tests (APHA, 2002). *Salmonella* spp. by pre-enrichment in 225 mL Buffered Peptone Water (Merck) at 37°C for 24 h; selective enrichment of 0.1 mL of pre-enrichment culture, in 10 mL of Rappaport-Vassiliadis (RV, Merck) broth for 24 h at 42°C; selective isolation by streaking 100 µL of RV in duplicate onto plates of Rambach agar (Merck) at 37°C for 24 h.

Physicochemical analyses

Total N and fat analyses were carried out using macro-Kjeldahl method (conversion factor 6.38) (IDF, 1993) and Gerber Method (IDF, 1997), respectively. Cheese moisture was determined using a forced-air, oven drying method at 100°C for 24 h (AOAC, 2000). Muffle furnace was used for ash determination and the pH determination was measured with a glass electrode (Model BT-600, Boeco) at ambient temperature (IAL, 2008). The total solids were calculated using Fleischmann's formula (MAPA, 2006). Non-fat solids were calculated as a percentage by subtracting the percentages of total solids from fat content (Brito *et al.*, 2006).

Culture settling plate technique at processing areas

Open Petri dishes containing 20 mL of Plate Count Agar media were distributed at the processing areas and exposed for about 15 min. The Petri dishes were closed and incubated at 35°C for 48 h. Results of mesophilic aerobic bacteria were expressed as CFU/cm² per week (Sveum *et al.*, 1992; Salustiano *et al.*, 2003).

Table 1. Microbiological counts in ovine and caprine dairy products.

Samples	Total Coliforms (log MPN/g)	Fecal Coliforms (log MPN/g)	Coagulase Positive Staphylococci (log CFU/g)	<i>Salmonella</i> spp. (in 25g)	Mesophilic Aerobic Bacteria (log CFU/g)
Legislation Standards for Low Moisture Cheeses ^(a)	3.00(b)	2.70(b, c)	3.00(b, c)	Absence in 25g ^(b, c)	-
Fascal's Curd	2.04±0.00	2.04±0.00	4.45±0.25	Absence	-
Fascal Cheese (1 day of ripening)	2.20±0.10	1.77±0.41	3.89±0.07	Absence	-
Fascal Cheese (90 day of ripening)	0.95±0.25	0.54±0.04	3.61±0.12	Absence	-
Serra da Estrela--type Cheese	1.50±0.03	1.39±0.04	1.95±0.92	Absence	-
Roquefort-type Cheese	1.50±0.03	0.85±0.20	N.D. ^(e)	Absence	-
Feta-type Cheese	1.63±0.29	0.72±0.14	N.D. ^(e)	Absence	-
Goat Feta-type Cheese	1.50±0.03	0.79±0.00	3.38±0.09	Absence	-
Legislation Standards for Raw Milk ^(a)	-	-	-	-	6.88 ^(d)
Ovine Raw Milk	2.04±0.00	2.04±0.00	4.28±0.36	Absence	4.30±0.43

Data are presented as mean ± standard error of the mean. ^(a)Maximum values accepted by the Brazilian legislation for cow milk: ^(b)MAPA, 1996, ^(c)ANVISA, 2001, ^(d)MAPA, 2002. ^(e)N.D.: Not detectable in 10⁻¹ dilution.

Swabbing method in surfaces

Sterile swabs (J. Prolab, São Paulo, Brazil) were moistened in 10 mL of sterile saline and moved over the surface using a standardized procedure (Evancho *et al.*, 2001). Swabbing was repeated using a dry swab and both swabs were then broken into the tube containing physiological sterile saline, which was vortexed for 10 s. Samples of manipulators were collected by swabbing the skin surfaces. The appropriate dilutions were plated on PCA media (drop plate, in triplicate). After drops had dried, plates were incubated at 35°C, for 48 h.

Statistical analysis

Microbiological and physicochemical analyses were done in duplicate. ANOVA model was used and treatment comparisons were performed using Student-Newman-Keuls test. All statistical analyses were evaluated to assess statistically significant differences at P<0.05.

Results and Discussion

Microbiological characterization of cheese samples

Mesophilic aerobic bacteria were enumerated in samples of raw ewe milk and the mean value observed was 4.30 log CFU/g (Table 1). This mean is below the maximum value (6.88 log CFU/g) accepted by Brazilian legislation standards for raw cow milk (MAPA, 2002). Considering the value for mesophilic bacteria of 5.70 log CFU/g, settled by the European

Community for ovine raw milk, the milk analyzed in this research presented lower counts than this maximum accepted (Buseti, 2006). Regardless the maximum values are not defined in raw ovine milk, the counts of coagulase positive staphylococci of 4.28 log CFU/g can be considered high. This bacterium is commonly involved in mastitis, a disease of primarily importance in dairy sheep and goats (Bishop and Morris, 2007).

Variability in microbiological counts between cheeses produced with sheep or goat milk is shown in Table 1. The absence of *Salmonella* and similar counts of coliforms were observed in both Feta-type and goat cheeses, although the presence of staphylococci (3.38 log CFU/g) was detected in the cheeses manufactured with goat milk.

Counts of total coliforms, fecal coliforms and coagulase positive staphylococci decreased during ripening of Fascal cheese. The microbiology of cheese ripening is frequently characterized by successions of communities. Coda *et al.* (2006) observed that whereas a large part of primary starter biomass declines throughout ripening, nonstarter lactic acid bacteria increase in hygienically produced raw milk cheeses. These bacteria may act as bioprotective cultures to inhibit pathogenic and spoilage microorganisms in cheese during the ripening process. Lactic acid bacteria grow at low temperature, and tolerate the lack of fermentable carbohydrates, low pH and aw, and the production of organic acids and bacteriocins by these bacteria make the environmental conditions

Table 2. Physicochemical analysis in ovine and caprine dairy products.

Samples	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Moisture (g/100g)	Total Solids (g/100g)	Non-fat Solids (g/100g)	pH
Ovine Raw Milk	5.7±0.1	6.7±0.5	0.7±0.1	82.4±0.5	17.5±0.6	10.8±0.1	6.27±0.08
Fascal Curd	5.7±0.2	6.0±0.1	0.6±0.1	83.4±0.9	-	-	4.75±0.01
Fascal Cheese (1 day ripening)	17.1±0.6	23.0±0.8	2.0±0.0	56.8±2.3	-	-	5.17±0.04
Fascal Cheese (90 day ripening)	24.6±0.1	33.7±0.2	2.5±0.2	31.9±1.2	-	-	5.23±0.03
Serra da Estrela- type Cheese	26.0±1.0	32.4±1.2	3.4±0.2	34.9±0.8	-	-	4.90±0.06
Roquefort-type Cheese	22.6±0.8	28.8±0.9	3.5±0.3	41.3±0.7	-	-	6.40±0.02
Feta-type Cheese	16.6±0.5	34.2±1.0	2.8±0.5	44.3±0.7	-	-	5.13±0.04
Goat Cheese	18.8±0.5	37.3±0.9	4.5±0.3	37.6±0.4	-	-	5.89±0.02

Data are presented as mean ± standard error of the mean.

very hostile during ripening (Nespolo and Brandelli, 2010).

All cheeses showed low counts of total and fecal coliforms (Table 1), within the limits established for Brazilian legislation (ANVISA, 2001), and lower than those reported for other ewe's cheese (Almeida *et al.*, 2007; Mucchetti *et al.*, 2008). Detection of total coliforms, fecal coliforms and *Escherichia coli* in Portuguese and Spanish ovine cheeses were extremely variable, ranging from 2 to 8 log CFU/g (Freitas and Malcata, 2000), in general higher than those observed in this work. In raw milk cheeses produced in Belgium, 31% showed coliforms and *Staphylococcus aureus* counts exceeding the standard values, reaching 7.8 and 6.0 log CFU/g, respectively (Vivegnis *et al.*, 1998).

On the other hand, values observed for coagulase positive staphylococci (Table 1) were greater than those established for Brazilian legislation (ANVISA, 2001) in Fascal and goat cheeses. For these cheeses, results were similar to those reported in fresh curd of raw milk Manchego cheese (Freitas and Malcata, 2000). Hard and soft Portuguese cheeses manufactured with ewe or goat milk also showed a variation in coagulase positive staphylococci counts (Almeida *et al.*, 2007). Fascal cheese ripened for 90 d presented lower microbiological counts compared with the same cheese after one day of ripening, but coagulase positive staphylococci count overrun the limit established by legislation.

Salmonella spp. was not detected in 25 g, for all cheese samples. Similar results were described for Vastedda cheese (Mucchetti *et al.*, 2008) and for most of the cheeses manufactured with ovine milk in the Iberian Peninsula (Freitas and Malcata, 2000). However, this pathogen was detected in samples of

Portuguese raw milk cheeses (Almeida *et al.*, 2007), Belgian raw milk cheeses (Vivegnis *et al.*, 1998) and French goat's raw milk cheeses (Kousta *et al.*, 2010). *Salmonella arizonae* was the only species of *Salmonella* detected in La Serena cheese (Freitas and Malcata, 2000).

Ripening time and the controlled storage temperature of cheese together with the intrinsic properties such as pH, aw, and presence of antimicrobial compounds produced by starter culture may contribute to decrease the risk from the pathogenic bacteria during cheese processing (Kousta *et al.*, 2010).

Physicochemical characterization of cheeses samples

The chemical parameters for ovine raw milk are showed in Table 2. Protein content was higher than those previously observed in milk obtained from sheep breeds in Brazil, such as 4.5 g/100g for Lacaune (Brito *et al.*, 2006) and 4.9 g/100g for Bergamácia (Sá *et al.*, 2005). However, the protein content was similar to that observed for Pampinita breed in Argentina (Suárez and Busetti, 2006). Values observed during the lactation period of Lacaune sheep often range from 4.9 from 7.0 g/100g (Brito *et al.*, 2006; Park *et al.*, 2007).

Total solids and non-fat solids in milk were 17.5 and 10.8 g/100 g, respectively (Table 2). These values were comparable to 16.2 and 10.4 g/100g, as calculated for Lacaune ovine milk at 140 days of lactation (Brito *et al.*, 2006). Total solids values observed in Bergamacia sheep milk was ca. 15 g/100g (Sá *et al.*, 2005), while higher values (20-24 g/10 g) were detected in Pampinita sheep milk (Suárez and Busetti, 2006).

The pH values of ewe cheeses ranged from 4.75 (Fascal curd) to 6.40 and the pH value of goat Feta-type cheese was 5.89 (Table 2). In Portuguese cheeses produced with ewe's or goat's milk, mean pH value was 5.5 for soft or hard cheeses (Almeida *et al.*, 2007). Large differences were found regarding the pH values 4.68 to 5.80, in Italian ewe cheeses (Coda *et al.*, 2006). In other investigation about Italian ewe cheeses, the pH values ranged from 5.0 to 5.04 (De Angelis *et al.*, 2001).

Aging time had the largest impact on composition of Fascal cheese, significantly affecting the changes in chemical parameters ($P < 0.05$) (Table 2). The pH values differed from the fresh curd until 90-d ripened Fascal cheese, and ranged from 4.75 to 5.23 during the ripening period. Cheeses at the end of the ripening presented pH values similar to other ewe's cheeses (Mendia *et al.*, 2000; Rizzello *et al.*, 2005; Coda *et al.*, 2006; Fallico *et al.*, 2006). The moisture values in Fascal cheese samples varied greatly from 31.9 to 83.4 g/100g, depending on the ripening period (Table 2). The final value for Fascal cheese (31.9 g/100g) was similar to that cited for Canestrato Pugliese cheese ripened for the same period (De Angelis *et al.*, 2001), and lower than those described for Italian and Spanish ewe cheeses (De Angelis *et al.*, 2001; Mendia *et al.*, 2000; Fallico *et al.*, 2006). Protein content increased during Fascal cheese ripening because of the significant decrease in moisture content (Table 2). The mean final value of protein was 24.6 g/100g, higher than those observed in Piacentinu Ennese (Fallico *et al.*, 2006) and Idiázabal (Mendia *et al.*, 2000), all cheeses ripened by equivalent periods. The Fascal cheese presented a fat content of 33.7 g/100g at the end of ripening (Table 2). The results were similar to those observed in some Italian ewe cheeses (De Angelis *et al.*, 2001), however much higher than the fat content in Vastedda cheese (Mucchetti *et al.*, 2008).

The pH value in Serra da Estrela-type cheese was 4.90 after about 80 d ripening. The variation of pH during the ripening of Serra da Estrela cheese, from 6.62 (fresh) to 4.84 (30-d ripened) has been described (Macedo *et al.*, 1993). At low pH, the free fatty acids are mainly in their protonated forms, thus causing a stronger impact on aroma (Tavaria *et al.*, 2004). Contents of fat and moisture were 32.4 and 34.9 g/100g, respectively; both values were comprised in the intervals observed for Serra da Estrela cheeses (Macedo *et al.*, 1993), although those authors reported maximum values of 23.0 g/100g for protein content.

Contents of protein, fat, dry matter and ash for Roquefort-type cheese were 22.6, 28.8, 58.7 and 3.5 g/100g, respectively. For the same parameters,

Raynal-Ljutovac *et al.* (2008) related 19.0, 33.0, 57.0 and 6.0 g/100g, respectively. The pH value of Roquefort-type cheese was 6.40, similar to those of Roquefort cheese at the end of maturation (FSANZ, 2005). Considering the moisture content in the Roquefort-type cheese and the fact that *Listeria* may grow if the pH rises to values near pH 6 (Gandhi and Chikindas, 2007), the presence of this bacterium should had been determined.

Evaluation of physicochemical properties of Feta cheese, realized by Prasad and Alvarez (1999) demonstrated higher contents of moisture (47-49 g/100g) and lower contents of fat (24-26 g/100g) than those observed in the Feta-type cheese analyzed in this work (Table 2). Although the pH value was similar, total protein and protein in dry matter were also lower when compared to those previously reported for traditional Feta cheeses, which ranged from 16 to 19 g/100g (Prasad and Alvarez, 1999; Moatsou *et al.*, 2002).

Values observed in the goat cheese were similar than those observed in 58-d ripened Ste Maure cheese (France), 90-d ripened Majorero cheese (Spain), 90-d ripened pressed cheese (Greece) and 45-d ripened pressed cheese (Spain) (Raynal-Ljutovac *et al.*, 2008).

Because their high fat values, all cheeses could be classified as fatty cheeses. Fascal and Serra da Estrela-type cheeses ripened for 90-d may be classified as low moisture or hard cheeses. Roquefort-type, Feta-type and goat cheeses presented higher moisture content, being classified as medium moisture or semi-hard cheeses.

Microbiological monitoring of water and processing areas

Microbiological monitoring of water in the dairy plant is shown in Table 3. Results for fecal coliforms below the detection limit of MPN Table, while the number of positive results was zero for all dilutions. Standards for potable water establish the absence of fecal coliforms (ANVISA, 2004) and no positive result was verified in these samples.

Counts of mesophilic aerobic bacteria at different processing areas, carried out by open plate technique demonstrated that contamination was higher in the milk reception area (Table 3). The counts exceeded the maximum value fixed by APHA (Sveum *et al.*, 1992). In cheese manufacturing and cheese molding areas, the values were below the limits allowed for processing areas. Salustiano *et al.* (2003) verified the microbiological air quality in a dairy plant and similar results were observed, considering related processing areas. Determination of mesophilic aerobic

Table 3. Microbiological monitoring counts at processing areas and of water in a dairy plant.

Samples	Fecal Coliforms (MPN/g)
Legislation Standards for Potable Water	Absence in 100 mL ^(a)
Water – Cheese Manufacturing	< 0.3
Water – Cleaning of Bulk Milk Tank	< 0.3
Mesophilic Aerobic Bacteria (log CFU/cm ² week)	
Maximum values for processing area air	1.48 ^(b)
Milk reception	1.83±1.10
Cheese manufacturing	1.35±0.57
Cheese molding	1.40±0.52
Mesophilic Aerobic Bacteria (log CFU/cm ²)	
Maximum values for processing area surface	0.30 ^(b)
Bulk Milk Tank	0.91±0.013
Mixing Tank ^(c)	0.13±0.001
Cheese Mould ^(c)	0.35±0.001
Mesophilic Aerobic Bacteria (log CFU/cm ²)	
Cheese Handler ^(d)	2.31±1.79

Data are presented as mean ± standard error of the mean. ^(a)Maximum values accepted by the Brazilian legislation: ANVISA, 2004; ^(b)Maximum values recommended by APHA (Sveum *et al.*, 1992); ^(c)Sampled were realized after cleaning, previous to the cheese

bacteria by sedimentation technique showed higher counts than those observed by culture settling plate technique (Salustiano *et al.*, 2003). The evaluation of microbiological quality of food service companies showed less than 20% of the air in processing areas was in good hygienic conditions, considering mesophilic aerobics (Andrade *et al.*, 2003). The cooling of room air in a Turkish cheese production plant had no effect on the total aerobic mesophilic bacteria counts but it had a significant effect on the increase of yeast and mold counts (Temelli *et al.*, 2006).

Samples collected from bulk milk tank presented the greatest counts and this equipment was located in the milk reception area. Lower counts were observed in mixing tank and cheese mould after cleaning (Table 3). Mixing tank was the only equipment in good hygienic conditions, according to APHA recommendations (Sveum *et al.*, 1992). In food service establishments, results of mesophilic aerobic counts showed that 19% of the equipment and utensil surfaces were in good hygienic condition (Andrade *et al.*, 2003). Cheese vat, cheese cloth and curd cutting knife were the contamination source of total aerobic mesophilic bacteria during Turkish cheese production (Temelli *et al.*, 2006).

Counts performed by swabbing in surfaces showed higher values in the skin of cheese handlers (Table 3). As there is no microbiological specification or recommendation for food handlers, Andrade *et al.* (2003) established five ranges of microbiological counts per hand. For mesophilic aerobic bacteria, 20.6% of samples were comprised within the range 2.00 to 3.00 log CFU by hand and these values

were considered acceptable by the authors. Results of the microbiological analyses of the samples collected from worker's hands during Turkish white cheese production indicated high counts of aerobic mesophilic bacteria, until 5.04 log CFU/cm² (Temelli *et al.*, 2006). Contamination of milk and cheese with *S. aureus* by food handlers has been reported (Kousta *et al.*, 2010).

Conclusions

Cheeses manufactured with different protocols showed variability in microbiological and chemical parameters. Ovine cheeses ready for consumption demonstrate microbiological and physicochemical quality in accordance with Brazilian legislation, and goat and Fascal cheeses exceeded counts for coagulase positive staphylococci. Good microbiological quality was also observed in water and milk samples, whereas elevated counts were observed in milk reception and storage areas. These results could be useful to establish a standard to the products and to the cheesemaking technology for manufacture of these regional cheeses.

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