Artigo Original/ Original Article

Microbiological and physicochemical characterization of the raw milk and the colonial type cheese from the Northwestern Frontier region of Rio Grande do Sul, Brazil

Caracterização microbiológica e físico-química de leite cru e queijo colonial da região Fronteira Noroeste do Rio Grande do Sul, Brasil

RIALA6/1659

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Recebido: 15.04.2015 - Aceito para publicação: 14.07.2015

ABSTRACT

This study aimed at evaluating the microbiological and physicochemical characteristics of raw milk and colonial type cheese from Northwestern Frontier region of Rio Grande do Sul, Brazil. For this purpose, the samples were collected in January and July. Microbiological analyses (aerobic mesophilic bacteria, total/thermotolerant coliform, coagulase-positive *Staphylococcus*, *Salmonella* spp. and *Listeria monocytogenes*, lactic acid bacteria) and physicochemical assays (pH, acidity, total solids, protein, fat, a_w, moisture, NaCl) were performed. The milk and cheese samples showed low microbiological quality because high counting of aerobic mesophilic bacteria, total/thermotolerant coliform and coagulase-positive *Staphylococcus* were detected. High counting of lactic acid bacteria was observed. However, neither *Salmonella* spp. nor *Listeria monocytogenes* was found. The standard deviations above one (1.0) in the fat, protein, moisture and salt contents indicated that no standard procedure was followed for producing the local cheese. The sample collection period caused differences in the microbiota, total solids of milk and cheese moisture contents, a_w and salt. The maturation period did not significantly influenced on the microbial counts, but it provided an increase in protein contents and a decrease in a_w value in cheese samples collected in July.

Keywords. sanitary conditions, microbiota, quality.

RESUMO

Este estudo avaliou as características microbiológicas e físico-químicas de leite cru e queijo colonial da região Fronteira Noroeste do Rio Grande do Sul, Brasil. Para isto, foram feitas coletas de amostras em janeiro e julho. Análises microbiológicas (bactérias aeróbias mesófilas, coliformes totais e termotolerantes, *Staphylococcus* coagulase positiva, *Salmonella* spp. e *Listeria monocytogenes*, bactérias ácido lácticas) e físico-químicas (pH, acidez, sólidos totais, proteína, lipídeos, A_a, umidade, NaCl) foram realizadas. As amostras de leite e de queijo indicaram baixa qualidade microbiológica, pois houve detecção de altos níveis de bactérias mesófilas, coliformes totais e termotolerantes e *Staphylococcus* coagulase positiva. Altas contagens de bactérias ácido láticas foram observadas. Entretanto, não foi detectada a presença de *Salmonella* spp. e *Listeria monocytogenes*. O desvio padrão acima de um (1,0) nos conteúdos de lipídeos, proteínas, umidade e sal indicou que não houve seguimento do procedimento padrão estabelecido na produção local de queijos. O período de coleta de amostras resultou em diferenças nas análises de microbiota, sólidos totais do leite e dos queijos, o teor de umidade, A_a e sal. O período de maturação não causou significativa influência sobre as contagens microbianas, mas promoveu aumento no conteúdo de proteína e diminuição na A_a dos queijos coletados em julho.

Palavras-chave. condições sanitárias, microbiota, qualidade.

INTRODUCTION

The State of Rio Grande do Sul is currently the second largest producer of milk from Brazil. The annual production of more than 3.6 billion liters represents 12 % of the national production. The Northwest Frontier region consists of 20 municipalities, 11.5 thousand milk producers and 40 thousand people directly involved in the dairy market. More than 1,062,744 liters of milk are produced and marketed per day, which corresponds to 15.94 % of the State's dairy production[1].

The milk is processed both, in large dairy industries as in small agribusinesses and transformed into different products. Among the products of milk agro-industrialization it is the cheese production, because it is a product with increased conservation time and that adds value to the raw material.

In Brazil, it is permissible to sell cheese from pasteurized milk or raw with maturation period of more than 60 days (artisanal cheeses). An exception occurs with the cheese manufactured in dairies from Geographical Indication registered region, in which the maturation period may be less than 60 days[2,3]. Although illegal, there is consumption of cheeses from milk without official sanitary inspection. Some dairy farmers sell part of their production to the industry and with the over plus they produce artisanal products on the property, which are destined for family consumption or marketing. Other producers turn all the milk manufactured into artisanal dairy products, allocating them to trade in property, free-trade or in small shops[4], among the artisanal products, the cheeses stand out.

Artisanal products without sanitary inspection can become a hazard to consumers, as well as spoilage microorganisms can serve as a vehicle for pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* spp.[5]. Several published researches in Brazil have shown the microbiological low quality of milk and cheeses marketed, characterized by frequent contamination of total coliform, *E. coli*, *S. aureus*, *Salmonella* spp. and *Listeria* spp [6–9].

In the microbiota of milk and artisanal cheeses, the lactic acid bacteria (LAB) are also present. They are inherent of raw material and can be responsible for sensorial characteristics of the artisanal cheeses[10]. LAB are Gram-positive, catalase-negative, non-sporingform and facultative anaerobic bacteria. According to the definition, LAB are bacteria able to ferment carbohydrate to lactic acid, mainly. These microorganisms even produced others antibacterial compounds including organic acids, ethanol, hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins[11].

Due the production of these compounds, several LAB showed antimicrobial activity against spoilage and pathogenic bacteria such as *Listeria* spp., *Staphylococcus* spp., *Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., and bacteria from coliform group[10,12–18].

The microbial groups of each milk product also vary according to geographical region where it is produced and they could be attributed to variations ratio of the utilized milk, predominating weather and processed methods used[5]. As there is no standardization in the manufacturing process as the coagulation time, type of rennet used, pressing, salting and moisture content of the final product and the great diversity of natural microbiota, it is possible to find in the market a variety of artisanal cheeses which, besides being illegal do not follow any identity and quality standard[19].

The knowledge of fabrication techniques has been transferred verbally through generations. By being fabricated, in most cases, with raw milk and without the addition of lactic ferments, a diverse microbial group undesired from the milk itself and also the low sanitary conditions in which is produced. In the Northwest Frontier region of Rio Grande do Sul this cheese is called colonial. The production of the colonial cheese is homemade (artisanal) and the marketing is informal.

The use of regional products, for its quality (unique flavours) and notoriety has become a habit among the population. With the increase in demand began to appear the fakes and the need for protection of the product, with the creation of laws.

In the current scenario commercially competitive, Geographical Indication (GI) can become a differentiator for the marketing, promoting the opening of new markets[20]. There are several cheeses with geographical indication in the world, such as Roquefort cheese, among others[21]. In Brazil, cheeses type Serrano, Coalho, Serro, Alto da Paranaíba, Canastra e Araxa with GI are stand out[22,23].

It is important the microbiological and physicochemical characterization of milk and colonial cheeses produced in the North West Frontier region as well as meet the historical tradition and the manufacturing practices. This knowledge can enable improve the milking practices, production and storage of the milk, adding value and special qualities.

Because of this, it was aimed to proceed with the microbiological and physicochemical evaluation of raw milk and colonial cheeses produced in different times of the year in the North West Frontier Region of RS/Brazil in order to add value to the products of this region with the Geographical Indication.

MATERIAL AND METHODS

Samples

Ten units of farming family system were selected from seven towns in the North West Frontier Region of RS/Brazil. They were pointed out by EMATER/RS, since they produce cheese from raw milk and do not added dairy ferment.

Each unit was collected: one (01) sample of fresh milk and two (02) cheeses from the same batch, weighing approximately 500 g, with 01 day of manufacture. A sample of cheese was analyzed immediately, characterizing the Time 01 (T1). The other sample of cheese was aged for seven days under refrigeration to 7 ºC, featuring Time 07 (T7). The choice of this time of maturation was determined from interviews with producers that reported this is the period in which the cheese was ripened before consumption/marketing. In each unit of production, there were two collections, one in January 2011, featuring the summer season, with temperature between 25 and 40 ºC, and another in July 2011, featuring the winter, with temperatures between 2 and 15 °C. In total, 20 samples of milk and 40 samples of cheeses were collected.

Microbiological characterization

Microbiological analysis for enumeration of total and thermotolerant coliforms, coagulase-positive *Staphylococcus* counts (CPS), aerobic mesophilic bacteria (AMB), lactic acid bacteria (LAB) and *Salmonella* spp., were performed on samples of milk and cheese, as recommended by the APHA[24]. All media used was from Oxoid (Uniphat Ltda, Basingstoke from England).

LAB were isolated after subculturing on MRS agar, and posteriorly checked for Gram reaction, catalase activity and examined microscopically. Only Gram-positive and catalase-negative isolates were further studied of bacteriocinogenic potential.

For the *Listeria* sp. analysis the DuPont™ Lateral Flow System *Listeria* test was used. When there were positive results to *Listeria* sp. on the kit, biochemical tests such as catalase test, motility at 25 ºC and 37 ºC, and performance of carbohydrate metabolism tests (API® 50 CH – Biomérieux) were performed to the confirmation of species.

Physicochemical characterization

All physicochemical analyses of pH, total acidity, moisture content, total solids, total fat, protein content, salt content (NaCl) and water activity were determined according to AOAC[25]. All analyses were carried out in triplicate.

The pH was measured directly with a pHmeter DM (Digimed – Brazil). The total acidity and NaCl were determined through titration methods. Moisture and total solids were determined by measuring the mass of food before and after the water was removed by evaporation. The water activity was measured instrumentally using a Water Activity System TESTO model 650.

RESULTS

Microbiological characterization

Table 1 shows the evolution of total and thermotolerant coliforms, CPS, AMB and LAB counts during the months of January and July. The analyses were performed on samples of milk and colonial cheeses.

The results of this study showed the AMB counts, on average, above 6 Log CFU.mL^{-1} in both samples of milk and cheeses. In Table 1, it can be seen that both samples of milk and cheese showed, on average, concentration greater than 3 Log MPN.mL^{-1} or g^{-1} total coliforms. Thermotolerant coliform, as determined in the milk samples, showed 1.4 and 0.36 Log MPN.mL^{-1} in January and in July, respectively. In samples of cheese, values, on average, above 2 Log MPN.g^{-1} of thermotolerant coliforms were observed. The milk, cheeses T1 and cheeses T7 collected in January showed 1.6, 2.19 and 1.72 Log CFU.g^{-1} of CPS, respectively. The samples of milk and cheeses T1 collected in July did not showed CPS counts.

The LAB counts, on average, they were above 5 Log CFU.mL^{-1}. In January as well as in July, a gradual increase in the LAB counts of milk comparing with cheeses samples was observed. After a count of LAB, 112 characteristics colonies from MRS plates were selected for morphological characterization and catalase test. Then, 62 isolates were confirmed such as Gram-positive and catalase-negative microorganisms. It was observed that 20 isolates showed cellular morphology of bacilli and 42 of cocci.

None of the samples showed the presence of *Salmonella* spp. and *L. monocytogenes*, neither in January or in July.

Physicochemical characterization

Analyzes of physicochemical characterization of raw milk samples were performed (Table 2). On average, the samples collected in January and July showed the following results: pH 6.70 and 6.68, total acidity 0.14 g/100 g lactic acid and 0.18 g/100 g lactic acid, total solids 11.31 g/100 g and 11.16 g/100 g, protein 3.26 g/100 g and 3.55 g/100 g and total fat 3.95 g/100 g and 3.58 g/100 g, respectively. Only results of samples from total solids were influenced by collection period.

Table 1. Microbial population in milk (Log CFU.mL^{-1}) and in artisanal cheese (Log CFU.g^{-1}) from the North West Frontier region of RS/Brazil during the months of January and July[1]

Microorganism	Collect period	Milk	Cheese T1*	Cheese T7**
Aerobic mesophilic bacteria	January	6.16 ± 0.59^{cB}	7.3 ± 0.43^{aB}	7.13 ± 0.81^{bB}
	July	6.51 ± 1.05^{cA}	7.53 ± 0.65^{bA}	7.95 ± 0.67^{aA}
Total coliform***	January	2.65 ± 0.67^{bB}	2.93 ± 0.22^{aB}	2.94 ± 0.19^{aB}
	July	2.96 ± 0.11^{bA}	$> 3,0^{aA}$	$> 3,0^{aA}$
Thermotolerant coliform*	January	1.4 ± 1.11^{cA}	2.44 ± 0.75^{aA}	2.21 ± 0.86^{bA}
	July	0.33 ± 0.51^{cB}	2.43 ± 0.86^{aA}	2.11 ± 0.91^{bB}
Coagulase-positive *Staphylococcus*	January	1.6 ± 2.08^{cA}	2.19 ± 2.94^{bA}	1.72 ± 2.84^{aA}
	July	ND^{bB}	ND^{bB}	$1,18 \pm 2,04^{aB}$
Lactic acid bacteria	January	5.93 ± 0.76^{cB}	8.14 ± 0.6^{bA}	8.33 ± 0.45^{aA}
	July	5.98 ± 1.52^{cA}	7.44 ± 0.74^{bB}	7.92 ± 0.82^{aB}

[1]Data are averages values ± standard deviations of the samples the two collections; a-c Means followed by the same letter in the row for each variable, do not differ at $p < 0.05$ significance by Tukey test; A-B Means followed by the same letter in the column for each variable, do not differ at $p < 0.05$ significance by Tukey test; *T1: Cheese with one days of aging; ** T7: Cheese with seven days of aging; ***Most probable number per milliliters (Log MPN.mL-1) and per gram (Log MPN.g-1); ND: Not detected

Table 2 showed the results of analyzes of physicochemical characterization of artisanal cheeses samples to protein, total fat, a_w, moisture and NaCl. On average, the samples of cheese T1 collected in January and July showed the following results: pH 6.05 and 6.20 and acidity titratable 0.30 g/100 g lactic acid and 0.41 g/100 g lactic acid, respectively. On average, the samples of cheese T7 collected in January and July, showed the following results: pH 6.02 and 6.00 and acidity titratable 0.37 g/100 g lactic acid and 0.42 g/100 g lactic acid, respectively.

DISCUSSION

The population of aerobic mesophilic bacteria (AMB) usually estimates the microbiological quality of dairy products. When present in quantities greater than 5 Log CFU.mL^{-1} it indicates deficiencies in hygienic production while concentrations lower than 4 Log CFU.mL^{-1} denotes good hygienic practices[5]. According to IN nº 62 of the Ministry of Agriculture, Livestock and Supply – MAPA[26], which regulates the standards for raw milk intended for the manufacture of pasteurized milk for direct human consumption or for processing into dairy products, this should present AMB count (called standard count on plates in that ordinance) of no more than 6 Log CFU.mL^{-1}.

The results of this study showed the milk inadequate hygienic conditions because the values of AMB, on average, above 6 Log CFU.mL^{-1} demonstrating that the milk used for cheese production was above the maximum stipulated by Brazilian legislation. In samples collected in July, only one of milk sample were within the standard AMB and in January, three were within the parameters established by legislation. The largest number of non-standard samples in July (winter) probably occurred because the producers have greater concern for the conservation of raw material/products in summer due to high temperatures, keeping them under-cooling. In winter, with lower temperatures, producers often retain the products at room temperature (between 2 and 15 ºC) which promotes the multiplication of microorganisms.

Table 2. Physicochemical characterization of samples of raw milk and artisanal cheeses from the North West Frontier region of RS/Brazil during the months of January and July[1]

Parameter	Collection period	Milk	Cheese T1*	Cheese T7**
pH	January	6,7 ± 0,06^{aA}	6,05 ± 0,41^{bA}	6,02 ± 0,36^{cA}
	July	6,68 ± 0,11^{aA}	6,2 ± 0,48^{bA}	6,00 ± 0,41^{bA}
Titratable acidity (%)	January	0,14 ± 0,01^{bA}	0,30 ± 0,11^{aA}	0,37 ± 0,07^{aA}
	July	0,18 ± 0,02^{bA}	0,41 ± 0,18^{aA}	0,42 ± 0,14^{aA}
Total solids (%)	January	11,31 ± 0,41^{A}	-	-
	July	11,16 ± 2,58^{B}	-	-
Protein (g/100g)	January	3,26 ± 0,35^{bA}	23.09 ± 4.03^{aA}	24.81 ± 4.03^{aA}
	July	3,55 ± 0,77^{cA}	19.49 ± 2.83^{bB}	22.98 ± 3.58^{aA}
Total fat (g/100g)	January	3,95 ± 1,17^{cA}	20.57 ± 3.68^{bA}	22.85 ± 3.06^{aA}
	July	3,58 ± 0,64^{cA}	21.50 ± 6.90^{bA}	24.21 ± 5.34^{aA}
a_w	January	-	0.945 ± 0.02^{aA}	0.939 ± 0.02^{aA}
	July	-	0.910 ± 0.03^{aA}	0.871 ± 0.03^{bB}
Moisture (g/100g)	January	-	47.53 ± 5,48^{aB}	41.73 ± 4.04^{bB}
	July	-	50.45 ± 3.66^{aA}	49.00 ± 3.14^{aA}
NaCl (g/100g)	January	-	4,58 ± 2,62^{aB}	4.36 ± 2.87^{bB}
	July	-	5.08 ± 2.49^{bA}	6.08 ± 3.58^{aA}

[1]Data are averages values ± standard deviations of the samples the two collections; a-c Means followed by the same letter in the row for each variable, do not differ at $p < 0.05$ significance by Tukey test; A-B Means followed by the same letter in the column for each variable, do not differ at $p < 0.05$ significance by Tukey test; * T1: Cheese with one days of aging; ** T7: Cheese with seven days of aging

The coliform group was used as another indicator of sanitary conditions for dairy products. Populations greater than 2 Log MPN.mL^{-1} indicate poor hygiene practices and environmental contamination[5]. In Table 1, it can be seen that both samples of milk and cheese showed an average concentrations greater than 2 Log or MPN.mL^{-1} or g^{-1} thermotolerant coliforms indicating, once again, the loss sanitary conditions of the raw material and final product.

The MAPA, through the ordinance nº 146[1], establishes a maximum value of 4 Log MPN.g^{-1} of thermotolerant coliform for high-moisture cheeses, such as those evaluated in this study. In the collection of January, six of cheese samples T1 and nine of T7 cheese samples were off the standard set by Brazilian legislation. In the collection of July, six of cheese samples T1 and two of T7 cheese samples were at odds with the established pattern.

Low counts of CPS in food are allowed, considering that only when their scores exceeded 5 Log CFU.g^{-1} or mL^{-1}, staphylococcal enterotoxins are produced, which are responsible for food poisoning caused by these microorganisms[28]. From the samples analyzed, three of cheeses T1 and two of cheese T7 collected in January and one of cheese T7 evaluated in July, with counts of CPS was above 5 Log CFU g^{-1}. These results indicated that these samples contained amount of cells able to produce enterotoxins at sufficient levels to cause staphylococcal intoxication. The ordinance nº 146 of MAPA[1] states that the maximum number of CPS presents in cheeses with high-moisture should be 3.7 Log CFU.g^{-1}. In the collection of January, four of cheese samples T1 and T7 three of the samples were not in accordance to the standards established by the Brazilian legislation, while the collection of July, only two of the samples of cheese T7 were not in accordance to the established standards. These results were relevant because, while the counts are lower than those necessary for the production of enterotoxins, temperature abuse during storage can provide an increase in the population of these microorganisms.

LAB are part of the natural microbial of the cow's udder and are present naturally in the environment milking and processing. Therefore, various bacteria that belong to the group of LAB can contaminate the raw materials and processing products[29]. Although being considered important spoilage in dairy products, the presence of LAB at high levels may interfere on the development of foodborne pathogens because they may have antimicrobial activity. Various LAB species are able to produce several substances with antimicrobial activity, such as organic acids, hydrogen peroxide, dyacetil, CO_2 and bacteriocins[11]. This may be one of the reasons why there was a lower counts of microorganisms hygiene indicators in the different samples when wild LAB with bacteriocinogenic potential are present[14,16,30]. The high LAB counts observed in this study may have contributed to the absence of *Listeria monocytogenes* and *Salmonella* spp. as well as to decrease the CPS counts in samples of cheeses collected in January.

An increase in the counts of the different microbial groups during the maturation period occurred in the samples of this study, except to thermotolerant coliform (January and July) and AMB (January). It is a normal phenomenon and results partially from microbial multiplication curdling and partially from the physical retention of these microorganisms in the curd when whey is run off[16]. The decrease in the counts of thermotolerant coliform can be explained due to the drop of water activity. This group needs 0.95 of water activity to grow at temperatures near the optimum growth temperature[29].

Despite the high counts of AMB and coliform group, none of the samples showed the presence of *Salmonella* spp. and *L. monocytogenes*. These results were of great importance, because they are pathogenic microorganisms of importance in foods. Similar results were obtained previously by Nero et al.[5] and Ortolani et al.[16] who observed a low pathogen incidence when microbiological quality of raw milk and cheeses were poor. These data indicated direct interference with the wild microbiota of animal origin foods, inhibiting the development and isolation of pathogens, as proposed by Jay[29].

Studies showed that milk and cheese produced/marketed in Brazil and worldwide, with microbiological quality deficit, were characterized by frequent contamination by pathogenic microorganisms and/or microorganism indicators at exceeding levels than those allowed by legislation[31-36].

Several factors influence the microbiota of dairy products, among them the season stands out. In this sense, Souza et al.[37] when studying the Serrano cheese observed that there was an increase of about one logarithmic cycle of milk for cheese coagulated mass when evaluating different microbial groups. The highest counts were reached after a week of maturation both in winter and in summer, decreasing during ripening. High scores were found AMB (6.07 Log $CFU.mL^{-1}$ in summer and 5.7 Log $CFU.mL^{-1}$ in winter) and a large number of total and thermotolerant coliforms in raw milk, indicating once again the deficient hygienic quality of raw material used in cheese processing. These microbial groups did not show significant variations in winter or summer, but the maturation time had a significant influence on their scores.

For physicochemical parameters of milk, the IN nº 62 of MAPA[26] establish that the minimum value protein and fat that raw milk should present is 2.9 and 3 g/100 g, respectively. Therefore, on average, the milk samples were within the standards established by legislation for the two parameters. The IN nº 62 of MAPA[26] determinate as 8.4 g/100g the standard for non fat solids in milk. There was significant difference in the values obtained between the two periods of sample collection. Analyzing the results, it was observed that in July, 70 % of the samples were not in accordance to the standards and in January, only 10 % were within the standards established.

As for acidity, the legislation stipulates that this should be between 0.14 and 0.18 g/100g lactic acid. In the collection performed in July, four of the samples were not in accordance to the standards set for this parameter, however, the collection performed in January, all samples were within standards set by that legislation. In January, temperatures ranged between 25 and 40 º C. Because of this, farmers have maintained their production stored in refrigerated environments. Possibly the measure adopted, avoided the proliferation of microorganisms which produce acid, justifying the acidity within the standards established by legislation in this period. Analyzing the results obtained, it was observed that in July, seven of the samples were not in accordance to the standards set in January and only one met the established limits.

Barbosa et al.[38], analyzed raw milk sold in the city of Queimadas/PA, which had 14 ºD of acidity, being within the acceptable standards for this parameter. In relation to fat content, the authors found, on average, 2.43 g/100 g of fat content that was below minimum allowed. About total solids, the samples were in disaccording with legislation because they showed, on average, 10.37 g/100 g.

The physicochemical analyses of the cheeses can be seen in Table 2. The collection period influenced the moisture and a_w of cheeses. The highest moisture values and a_w found in July were probably because the low temperatures and relative humidity was higher at this time, making the evaporation of water and removal of whey harder. It was also observed that the collection period did not significantly affect the pH of the cheese, but it significantly affected the values of acidity. It was a consequence of the lactic acid production by LAB[37].

The salt, conferring the taste characteristic or enhancing flavor complements syneresis and regulates the cheese acidity, favoring the release of free water in the mass by the difference in osmotic pressure and dissolution of some proteins and their products, all of which were substances titrated like acids. During the maturation, buffer substances were released, for example, casein, which is as titratable acid. The separation of casein whey was a factor that favors the variation of pH and acidity[39]. It may be noted, too, that the collection period significantly influenced the NaCl content of the cheeses. The standard deviation above one (1.0) showed that differences found between cheeses due to the fact that the amount of salt added was not measured.

Values of standard deviations above one (1.0) for fat, protein, salt and moisture allow interpreting that there was no standardization in the production of artisanal cheeses from the North West Frontier region, at both the technological steps (pressing, cutting, salting, etc.) as the composition and quality of the feedstock. In this sense, the difference observed in the composition of milk used for cheese production may have contributed to the variation observed in cheeses.

Delamare et al[4] analyzed licensed and unlicensed commercial Serrano cheeses, a traditional product from the highlands of South Brazil and their physicochemical characteristics obtaining, were corroborated with this study except to the content of total fat and NaCl.

Other study has shown that the season in which dairy products were produced also has an influence on their physicochemical characteristics. Souza et al[37] evaluated the influence of the seasons on the physicochemical composition of Serrano cheese and observed that there was a significant decrease in pH during the first week of maturation, both in winter (5.1) as in summer (5.3). However, in relation to water activity (a_w), there was a decrease pronounced in summer, probably due to large water evaporation and hydrolysis of proteins, caused by higher temperatures this time of year. The moisture of the cheeses varied significantly between the two periods, being lower in summer due to high temperature and the low relative humidity that caused an intense dehydration.

There was a relationship between development of microorganism and physicochemical parameters of the products. Foods with high pH, high water activity or low NaCl content are appropriated to growth at pathogens and spoilage microorganisms. This relationship can be observed in studies performed Souza et al[37], Kongo et al[34] and Dalla Rosa et al[40]. However, in this study, this was not observed because in July when there was lower pH, lower a_w, higher acidity and higher NaCl, the counts of coliform and aerobic mesophilic bacteria were higher than in January.

CONCLUSION

High counts of microorganism as *Staphylococcus* and coliforms indicated poor quality of the milk and artisanal cheeses, although *Salmonella* spp. or *L. monocytogenes* were not detected. The great variation in physicochemical characteristics of the cheeses indicates that there is not standardization of the raw material or the technological steps. The collection period resulted in differences in the microbiota, total solids of milk and cheese moisture content, aw and salt. The maturation period did not significantly influenced the microbial count, but it provided an increase in protein and decrease in a_w cheeses collected in July.

ACKNOWLEDGMENTS

Department of Science, Innovation and Technology Development of the State of RS. Reference term nº 0001/2009. Institutional Program of Scientific Initiation Scholarships Regional University of North West RS. Post-graduate Program in Food Science and Technology, Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas.

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