

CHANGES IN THE MICROBIOLOGICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF *SERRANO* CHEESE DURING MANUFACTURE AND RIPENING

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ABSTRACT

Changes in the microbial flora present in six *Serrano* cheese batches (3 ripened in Summer and 3 in Winter) were studied during production and ripening, to microbiologically characterize this cheese, which is traditionally manufactured by farmers in the south of Brazil, using raw cow milk, without the addition of starter cultures. The levels of several microbial groups were enumerated in the milk, curd and different stages of the 60-day long ripening period. Values of some physicochemical parameters, such as pH, titratable acidity, water activity, moisture and NaCl content were also determined. The correlation coefficients between these parameters and the microbial groups throughout ripening were determined. The influence of the season of the year was also studied. Results demonstrated that the microflora and the physicochemical characteristics of *Serrano* cheese changed significantly during the 60-day ripening period, especially in Summer. Almost all microbial groups reached their highest counts after the 7th day of maturation, steadily decreasing towards the end of the process. Higher microbial counts during the first week of ripening was accompanied by lactic acid production and, as a consequence, a sharp drop in the pH of cheese was observed. Lactic acid bacteria comprised the main microbial group present in *Serrano* cheese and their counts were similar to the total viable counts at all sampling points. The abundance of lactobacilli during the manufacture and ripening suggests that these microorganisms may play an important role in the production of *Serrano* cheese. In conclusion, this work shows the importance of ripening time being longer than 30 days to allow for microbiological stabilization of this artisanal product. Also, except for halotolerants and moulds counts, and for moisture content, there were no significant differences observed either for microbial counting or for physicochemical characteristics of the cheese concerning the season of the year in which the product was manufactured.

Key words: *Serrano* cheese, cheese ripening, food microbiology, lactic acid bacteria.

INTRODUCTION

As food markets expand and diversify, there is a growing interest in traditional dairy products manufactured at the farm level due to the uniqueness of such foods and the difficulties in mimicking them on an industrial scale. *Serrano* cheese, produced in the highlands of Rio Grande do Sul, south of Brazil, is a small scale industrial product with high acceptance by the consumer.

There is very little information available on the microbiology and physicochemical characteristics of artisanal Brazilian cheese. Presently, only the *Minas* cheese has been studied

(14,15). *Serrano* cheese is a farm-made variety manufactured from raw milk, in a region located in an altitude of 950 m above sea level and the average temperatures range from 4°C to 13°C in winter and from 18°C to 26°C in Summer.

Serrano cheese is an uncooked, semihard product, with a strong flavour. Its rind is thin, uniform and smooth, with a soft straw-yellow colour and the cheese bulk has a whitish colour with irregularly distributed holes. It is consumed after a short period of ripening, usually 30 days, although the present Brazilian regulation on food safety for cheeses made from raw milk states a minimum ripening period of 60 days prior to consumption (5).

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Although *Serrano* cheese is highly appreciated and consumed, its microbial flora and physicochemical characteristics are unknown. Such lack of knowledge is particularly critical, specially the microbiological quality, because this cheese is manufactured with raw milk and no commercial starters are used in its manufacture, fermentation occurring, therefore, by a natural process. Ripening depends entirely on natural microflora. These artisanal techniques, which include the use of raw milk, acidification by the indigenous lactic acid bacteria present in milk and absence of control of temperature and relative humidity in the maturation room, result in a lack of uniformity and quality of the final product.

The knowledge of microbiological and biochemical reactions in cheese can help to introduce process modifications necessary to improve the quality of products, without any loss in its characteristics. The aims of the present study were to investigate the changes in levels of the main microbial groups and in the physicochemical characteristics throughout the ripening process of *Serrano* cheese and the influence of the season of the year over these interactions. It is expected that this study will contribute to the establishment of microbiological quality standards for *Serrano* cheese, as well as to provide further scientific basis for the understanding of ripening process, which is necessary to improve farmhouse procedures.

MATERIALS AND METHODS

Cheese manufacturing

Serrano cheese is manufactured according to the following procedure. Whole raw milk is filtered and salted with 4-18g NaCl per litre of milk. Coagulation takes place at an average temperature of 35°C for 60-75 min by adding 4 g of commercial calf rennet (1: 65000 strength) per litre of milk. Once the right degree of consistency is attained, the curd is cut and the whey is drained at 35-37°C. Then, the curd is transferred into perforated plastic molds following whey drainage for 24 h under pressure. Cheeses are allowed to mature without any temperature or humidity control, with variables changing only with year season, in a special maturing house. During ripening they are inverted daily and washed with warm water once a week, with ripening lasting for as long as 30 days. Approximately 10L milk are used to obtain flat-cylinder shaped cheeses with 14-17 cm of diameter, 4-7 cm high and weighing around 800-1200 g.

Sampling

Six batches of *Serrano* cheese manufactured in three different dairies by experienced cheese makers following the traditional method, as described above, were studied. Three were manufactured in Summer (January), the others in winter (July). For every batch, milk, curd and cheeses of 7, 14, 28, 42 and 60 days of maturation were analyzed. Each sample consisted of one whole cheese. Sampling was performed according to

International Dairy Federation (IDF) Standard 50B (18). All samples were transported to the laboratory under refrigeration at 4°C and analyzed on arrival.

Microbiological analysis

Representative 25 g curd or cheese samples were homogenized with 225 mL of a sterile 2% (w/v) sodium citrate solution, previously warmed to 45°C, in a Stomacher Lab-Blender 400 (Seward Medical, England) for 1 min. Decimal dilutions were prepared in 90 mL of sterile 0.1% (w/v) peptone water and aliquots were plated in duplicate on a variety of media. Total viable count (TVC) and psychrotrophics were made on plate count agar incubated at 32°C for 48 h and 7°C for 10 days, respectively. Lactobacilli were enumerated on Rogosa agar acidified to pH 5.4 with acetic acid after incubation at 30°C for 5 days in an anaerobic jar (Oxoid, Unipath Ltd., Basingstoke, England) with anaerobiose produced by Anaerocult A (Merck, Darmstadt, Germany). Lactococci were determined on M17 agar incubated at 30°C for 72 h. Proteolytic and lipolytic organisms were enumerated on plate count agar supplemented with reconstituted skim milk at 10% and tributyrin agar respectively, after incubation at 32°C for 72 h. Moulds and yeasts were determined on potato dextrose agar acidified with 10 mL/L of 10% (w/v) sterile tartaric acid incubated at 25°C for 5 days. Halotolerants were enumerated using mannitol salt agar at 32°C for 72 h. Fecal and total coliforms were enumerated on violet red bile agar after incubation for 24 h at 44 and 32°C, respectively. The surface spread technique was used for psychrotrophics, proteolytic and lipolytic organisms, halotolerants and for moulds and yeasts. The pour plate technique was used for TVC. The pour plate and overlay technique was used for lactobacilli, lactococci and fecal and total coliforms. After incubation, plates with 25-250 colonies were counted. All microbiological analysis were performed according to American Public Health Association (1). All media were from Oxoid (Unipath Ltd., Basingstoke, England).

Isolation procedure and identification of lactic bacteria

For all sampling points, plates corresponding to dilutions of 25-50 colonies/plate were selected from M17 and Rogosa media. From each of these plates five to eight colonies, representing all different morphologies, were then picked at random. The isolates were purified by 4 cycles of subcultures on MRS broth and agar at 30°C and stored in MRS broth containing 20% (v/v) glycerol, at -12°C. Isolates were identified according to the criteria of Sharpe (28). In order to identify the genera several tests were applied: Gram staining, microscopic examination, catalase, growth at 10 and 45°C in MRS broth, salt tolerance (6.5% NaCl in MRS broth), L-arginine hydrolysis and production of carbon dioxide from glucose. These tests were performed according to techniques described by Harrigan and McCance (16).

Physicochemical analysis

The pH of the milk was measured directly with a pHmeter DM 20 (Digimed, Brazil) and the titratable acidity of milk was measured following the method 947.05 (3). The pH and titratable acidity of curd and cheese were measured as described by the methods 14.022 (2) and 920.124 (4), respectively. The moisture content and the water activity (a_w) were determined instrumentally using a MA30 Moisture Analyzer (Sartorius, Germany) and an Aqua Lab CX-2 Water Activity System (Decagon Devices, USA), respectively. The NaCl content was determined according to method 935.43 (4). All analyses were carried out in duplicates.

Statistical analysis

Plate count data were transformed into logarithms prior to statistical treatment. Statistical analysis was performed using the SPSS software (29). Differences between the treatment means were compared at the 5% level of significance. Data were subjected to analysis of variance (ANOVA) in order to assess the statistical significance of the effect of ripening time in the microbial counts and physicochemical parameters and the Tukey's or Dunnett T3

test were performed for comparison of means. Comparison of means by the t test was used to determine the influence of the season of the year on the microbiological and physicochemical characteristics of cheese. Statistical correlations between log counts of the major microbial groups and physicochemical parameters investigated were assessed by the Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Changes in the microbial groups counts during ripening

The counts of the different microbial groups investigated during the manufacturing and ripening of Serrano cheese are shown in Table 1.

An increase of around 1 log was found from milk to curd in the counts of the different microbial groups investigated, except for filamentous fungi. This increase in the number of microorganisms is a normal phenomenon in the manufacturing of cheese and it results partially from microbial multiplication during curdling and partially from the physical retention of these microorganisms in the curd when whey is run off. The low increase

Table 1. Changes in the counts (log cfu/g) of the microbial groups of *Serrano* cheese during manufacturing and ripening in summer¹ and in winter¹.

Microbial Group (log cfu/g)	Season	Milk	Curd	Ripening time (days)				
				7	14	28	42	60
TVC	Summer	6.07±0.64 ^a	7.60±0.90 ^b	9.32±0.05 ^c	9.00±0.05 ^{bc}	8.57±0.11 ^{bc}	8.36±0.44 ^{bc}	7.71±0.62 ^b
	Winter	5.70±0.77 ^a	7.40±1.15 ^{ab}	9.19±0.36 ^c	9.12±0.24 ^c	8.65±0.28 ^{bc}	7.98±0.43 ^{bc}	7.56±0.21 ^{bc}
Psychrotrophic	Summer	6.00±1.09 ^{ab}	7.35±0.70 ^{ab}	8.06±0.09 ^b	7.80±0.39 ^b	6.90±0.78 ^{ab}	6.72±1.34 ^{ab}	4.94±1.62 ^a
	Winter	5.49±0.95 ^a	6.49±0.54 ^{ab}	8.26±0.52 ^c	8.15±0.51 ^c	8.10±0.34 ^c	7.64±0.34 ^{bc}	7.02±0.56 ^{abc}
Proteolytic	Summer	5.03±0.33 ^a	5.91±0.27 ^{ab}	7.12±1.40 ^{ab}	8.09±0.34 ^b	7.50±0.88 ^b	7.17±0.58 ^{ab}	7.10±1.18 ^{ab}
	Winter	4.88±0.72	5.97±0.59	7.09±2.27	9.05±0.34	8.67±0.16	7.82±0.79	7.86±0.75
Lipolytic	Summer	3.94±0.92 ^a	5.18±0.57 ^a	6.50±0.74 ^a	6.59±1.67 ^a	6.43±0.99 ^a	5.57±0.74 ^a	5.50±0.93 ^a
	Winter	4.94±0.76 ^a	6.15±0.50 ^a	6.16±0.60 ^a	6.26±0.81 ^a	5.13±0.64 ^a	5.30±0.84 ^a	4.68±1.46 ^a
Lactococci	Summer	5.84±0.66 ^a	7.60±0.84 ^b	9.31±0.03 ^c	8.92±0.10 ^{bc}	8.42±0.20 ^{bc}	8.17±0.57 ^{bc}	8.00±0.31 ^{bc}
	Winter	5.63±0.48	7.32±1.31	9.14±0.30	9.07±0.43	8.29±0.14	7.70±0.38	7.68±0.31
Lactobacilli	Summer	4.32±1.43 ^a	5.62±0.89 ^a	8.38±0.63 ^b	8.34±0.38 ^b	8.39±0.21 ^b	8.27±0.27 ^b	8.15±0.04 ^b
	Winter	3.46±1.11	4.16±1.41	7.24±1.71	7.77±0.95	7.98±0.83	7.91±0.37	7.92±0.21
Halotolerant	Summer	5.26±0.57 ^{ab}	6.42±0.56 ^b	6.52±0.56 ^b	5.72±0.01 ^{ab}	5.24±0.42 ^{ab}	4.63±0.78 ^a	4.64±0.79 ^a
	Winter	4.55±0.58 ^a	6.13±0.67 ^b	6.74±0.14 ^b	6.37±0.57 ^b	6.09±0.43 ^b	6.15±0.26 ^b	6.02±0.03 ^b
Moulds	Summer	2.49±0.27 ^a	2.53±0.21 ^{ab}	4.70±0.30 ^b	3.67±0.06 ^{ab}	2.93±0.83 ^{ab}	1.20±2.08 ^a	1.94±1.68 ^a
	Winter	1.49±1.31 ^a	1.49±1.29 ^a	2.52±0.91 ^a	2.64±0.59 ^a	1.43±1.25 ^a	1.33±1.15 ^a	0.67±1.15 ^a
Yeasts	Summer	3.03±0.77 ^a	4.42±0.76 ^{ab}	6.04±0.27 ^b	4.81±0.70 ^{ab}	4.80±0.55 ^{ab}	4.24±0.70 ^{ab}	3.72±0.66 ^a
	Winter	3.37±0.63 ^a	4.59±0.37 ^a	5.51±1.04 ^a	4.59±0.84 ^a	4.63±0.80 ^a	4.08±1.55 ^a	4.07±1.17 ^a
Total Coliforms	Summer	4.03±1.34 ^{ab}	5.57±0.86 ^b	5.76±1.18 ^b	5.45±1.33 ^b	4.07±2.37 ^{ab}	3.18±2.37 ^{ab}	0.57±0.88 ^a
	Winter	3.54±0.96 ^a	4.84±0.82 ^a	4.98±1.27 ^a	3.65±2.14 ^a	3.13±3.11 ^a	2.74±3.00 ^a	3.01±2.98 ^a
Fecal Coliforms	summer	3.13±1.00 ^{ab}	4.27±0.54 ^{ab}	5.59±1.24 ^b	5.29±1.26 ^b	4.00±2.37 ^{ab}	2.69±2.69 ^{ab}	<10 ^a
	winter	1.75±0.14 ^a	3.09±0.61 ^a	3.92±1.15 ^a	2.44±2.79 ^a	2.34±2.50 ^a	2.08±2.15 ^a	2.18±2.29 ^a

¹Data are the average values ± standard deviation of three batches; ²Total viable counts; ^{a-c}Means in the same row with different superscripts differ (P<0.05).

in the counts of all microbial groups of *Serrano* cheese may be explained by the short time interval between the collection of milk and curd samples (approximately 1.5 h), in which microbial multiplication is minimum. According to Tatini *et al.* (30), the physical retention of microorganisms in the curd allows for the 1 log increase in the counts, the remaining being due to microbial multiplication. For most microbial groups maximum counts were attained after 1 week of ripening, both in Summer and winter.

Significant differences in the counts of halotolerants and moulds were only detected in association to the season in which the cheeses were manufactured and ripened. For moulds, an increase in the counts was observed in the Summer production when compared to the winter, perhaps due to lower temperatures, which decreased the growth rate of this microbial population. For halotolerants a more pronounced decrease in Summer counts was observed, possibly due to environmental conditions, which is in agreement with the high coefficient of correlation ($r=0.732$) between a_w and halotolerants in this season.

In the raw milk mean counts of 6.07 and 5.70 log were achieved for TVC. These high counts are likely to stem from poor hygiene conditions during milking and are similar to those found in milk used for the production of other cheeses (7,11,13,24) but lower than those determined in milk used in the manufacturing of Tafi (10) and León cheese (21). The number of CFU increased during the first week of ripening, achieving counts around 9.2-9.3 log, which, despite being higher than those found for *Ulloa* cheese (24), are similar to counts found for other varieties (7,8,10,21,27). According to Kosikowski (19), values of 9 log are normal in cheese produced with raw milk. The coefficients of correlation found between pH values and the TVC in Summer ($r=-0.819$) and winter ($r=-0.827$) suggest that this microflora may be comprised mainly of acid-tolerant microorganisms, as suggests correlations found between these counts and the titratable acid values ($r=0.607$ and $r=0.647$, respectively).

The psychrotrophic counts in milk, 6.0 and 5.49 log, are similar to those found by other authors (13,21). A significant increase was observed in the number of these microorganisms in milk until the first week of ripening, attaining values similar to those of San Simón (13) and León (21) cheeses. From the maximum count achieved at the 7th day, a constant drop-off was observed until the end of the 60-day period. However, this reduction was more pronounced in Summer (3 log) in comparison with winter (1 log), possibly due to environmental conditions, which contributed to a quicker decrease in this microbial population; this is in agreement with the coefficient of correlation between a_w and these microorganisms in Summer ($r=0.548$). The high amount of psychrotrophics in *Serrano* cheese represents a potential risk of deterioration of this product. This problem becomes even more important as most cheeses are sold within 30 days of production and consumers store them at refrigeration temperatures. There is a significant difference between the psychrotrophic counts in the 60-day

cheese and that of younger cheeses showing that a 2-month ripening period is the key to a reduction of these counts.

The proteolytic counts were higher than the lipolytics, this may be an indication that proteolytic activity is greater than lipolytic activity in this cheese, which is common in this kind of short ripening period cheese (17). The coefficients of correlation attained between the titratable acidity values and the proteolytic counts in Summer ($r=0.733$) and in winter ($r=0.675$) suggest that this population may be mainly comprised of acid-tolerant microorganisms. The correlations obtained between these counts and the pH values of Summer samples ($r=-0.758$) and winter samples ($r=-0.701$) are in accordance with this hypothesis.

The counts of lactococci of the milk until the cheeses at 2 weeks were 10^1 to 10^3 times higher than those of lactobacilli, both being similar in the following stages due to a higher death rate of the lactococci population during the final phase. The same phenomenon was observed by Fontán *et al.* (13). Ordóñez and Burgos (24) observed an increase of lactobacilli during the ripening of *Ulloa* cheese and Tornadizo *et al.* (31) found that lactobacilli were the prevalent microbial group in the late ripening stages of *Armada* cheese. According to Peterson *et al.* (25), during the fermentative process of cheese the formation of several metabolites, such as lactate, citrate, glycerol, and amino acids, among others, which are better utilized by lactobacilli, takes place.

The halotolerant microflora found in milk and cheese (5-6 log) were similar to those of other cheese varieties, such as Casar de Cáceres (26), Arzúa (7), Tetilla (22) and San Simón (13). For León (21), Cebreiro (8), Afuega'l Pitu (9) and Anevato (17) cheeses, counts by 2-3 log units lower were found. These differences were probably due to pH variations, since these microorganisms are acid-sensitive. In this cheese and others that had a similar halotolerant microorganisms count, the pH was maintained around 5.0, favoring the development of this population. As these microorganisms were found in significant levels they might contribute to the ripening process to some extent, through their lipolytic and proteolytic activities, playing a role in the change in texture and aroma development. However, as halotolerants include mainly the *Micrococcaceae* family, which is comprised of the genera *Micrococcus* and *Staphylococcus*, one must pay heed to this result, since there is a potential risk for consumer health if enterotoxin-producing coagulase-positive *Staphylococcus aureus* are present.

The counts of moulds and yeasts during ripening were similar to those found in other cheeses, such as Tetilla (22) and Arzúa (7). As ripening advances, there is an increase in cheese matrix compaction, which reduces the diffusion of the oxygen required for the multiplication of these microbial populations.

The high amount of total and fecal coliforms detected in raw milk is again an indication of the low hygienic quality of the raw material used in the manufacturing of this cheese. These microbial groups did not present significant variations in winter while, in Summer, the time of ripening had a significant influence over the

counts of these populations and the 60-day ripening time was important for their reduction. An important feature regarding the sanitary-hygienic aspect is the absence of fecal coliforms in the end of the ripening process in Summer. The coefficients of correlation among the counts of total coliforms, a_w ($r=0.527$) and moisture content ($r=0.569$) show that these parameters influenced the decrease of this population in Summer. Total coliforms were not detected in Tafi cheese after 30 days of ripening due to low a_w (10). The higher temperatures at the maturation room in this season also showed influence over the coliform population. The 60-day old Manchego cheese ripened at 10°C had a count of 2.07 and 2.24 log for total and fecal coliforms, respectively; however, when ripening took place at 20°C, neither population was detected (23). Total coliforms had a higher decrease rate in La Serena cheeses ripened at 16.9°C than in those ripened at 13.6°C (12). The high levels of total and fecal coliforms may be the main cause for the formation of holes in Serrano cheese.

Lactic acid bacteria isolated during ripening

Of a total of 484 isolates in cheese, 431 were identified as LAB. Fig. 1 shows the distribution rate of lactic bacteria identified throughout the process of manufacturing and ripening.

The lactobacilli were the most abundant lactic bacteria, followed by enterococci and lactococci. The lactobacilli prevailed throughout the manufacturing and ripening process, especially in the late steps of the process, suggesting that these microorganisms play an important role in the production of this cheese and could be part of its lactic culture. Maybe the low ratio of lactococci and leuconostoc in the lactic isolates stems from adding salt to milk during cheese preparation, inhibiting the growth of these microorganisms. The low amount of

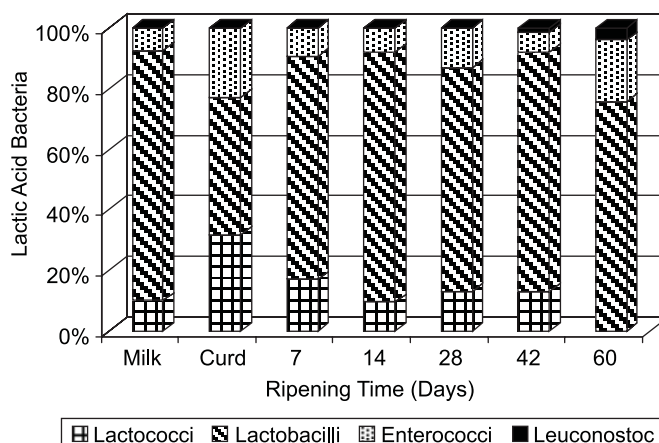


Figure 1. Evolution of genera of LAB (431 strains) during manufacturing and ripening of *Serrano* cheese. Percentage of strains of each genera isolated from M17 and Rogosa at each stage.

heterofermentative lactobacilli and *Leuconostoc* spp. supports the hypothesis that the great number of holes in this cheese results from the high count of coliforms.

Changes in the physicochemical characteristics during ripening

Table 2 shows the evolution of the physicochemical characteristics investigated during the manufacturing and ripening of *Serrano* cheese.

The pH around 6.7 in raw milk used for the manufacturing of this cheese is similar to that of milk used for the production of other cheeses made from bovine milk (7,9,11,13,21). During the

Table 2. Changes in the physicochemical characteristics of *Serrano* cheese during manufacturing and ripening in the summer¹ and in the winter¹.

Characteristic	Season	Milk	Curd	Ripening time (days)				
				7	14	28	42	60
pH	summer	6.71±0.05 ^c	6.36±0.19 ^{bc}	5.33±0.09 ^a	5.52±0.05 ^a	5.80±0.36 ^{ab}	5.75±0.33 ^{ab}	5.66±0.23 ^a
	winter	6.66±0.04	6.29±0.56	5.1±0.21	5.20±0.25	5.24±0.19	5.30±0.23	5.30±0.19
Titratable acidity ²	summer	0.2±0.0 ^a	0.2±0.0 ^a	1.1±0.1 ^b	1.1±0.2 ^b	0.9±0.3 ^{ab}	1.3±0.5 ^b	1.4±0.4 ^b
	winter	0.2±0.0 ^a	0.3±0.2 ^{ab}	0.9±0.2 ^{bc}	0.9±0.3 ^c	1.0±0.3 ^c	0.9±0.3 ^{bc}	1.0±0.2 ^c
Moisture (%)	summer	-	50.78±1.17 ^d	40.52±4.73 ^c	36.85±2.26 ^{bc}	36.63±2.82 ^{bc}	29.56±2.46 ^{ab}	27.57±0.72 ^a
	winter	-	57.30±3.76 ^d	45.37±1.71 ^c	43.34±0.88 ^{bc}	39.81±0.73 ^{bc}	36.80±1.06 ^{ab}	32.98±4.16 ^a
a_w	summer	-	0.987±0.003 ^c	0.97±0.004 ^{bc}	0.962±0.006 ^b	0.953±0.007 ^{ab}	0.939±0.010 ^a	0.935±0.007 ^a
	winter	-	0.984±0.005 ^b	0.970±0.004 ^{ab}	0.967±0.003 ^{ab}	0.966±0.006 ^{ab}	0.957±0.008 ^a	0.954±0.012 ^a
NaCl (% of TS)	summer	-	1.4±0.6 ^a	1.2±0.4 ^a	1.3±0.4 ^a	1.6±1.0 ^a	1.4±0.6 ^a	1.4±0.6 ^a
	winter	-	1.3±1.5 ^a	1.6±0.9 ^a	1.3±0.7 ^a	1.5±0.8 ^a	1.7±0.9 ^a	1.5±0.8 ^a
NaCl in moisture(%)	summer	-	1.4±0.6 ^a	1.9±0.9 ^a	2.3±0.9 ^a	2.6±1.3 ^a	3.1±1.3 ^a	3.4±1.3 ^a
	winter	-	0.9±0.9 ^a	1.8±1.0 ^a	1.7±1.0 ^a	2.2±1.1 ^a	2.8±1.6 ^a	3.1±1.8 ^a

¹ Data are the average values ± standard deviation of three batches; ² Expressed in % of lactic acid;

^{a-c} Means in the same row with different superscripts differ significantly ($P<0.05$).

curdling process, the pH decreased significantly during the first week of ripening, when it hit the lowest values in the whole process; 5.3 in Summer and 5.1 in winter. This decrease by approximately 1.5 units is a consequence of the lactic acid production by lactic bacteria. Manchego cheese ripened at 10°C and 20°C had pH values of 5.07 and 5.28, respectively (23). The significant increase in pH between the first and the fourth ripening week in Summer result from the metabolic activity of mould and yeast, which use lactic acid as a source of carbon, and/or the proteolytic process that releases great amounts of nitrogenated alkaline compounds (31). In winter, the pH did not present significant variations after the 7th day of ripening. The lower temperatures of this period are likely to have reduced the intensity of biochemical reactions, resulting in a constant pH until the end of the process. The pH values during the ripening, mainly in Summer, favored the action of microbial proteases, which present maximum activity in the pH interval between 5.5-6.5 (6).

The more pronounced decrease in a_w in Summer is probably due to the greater water evaporation and hydrolysis of proteins during ripening in this hotter temperature period. The mean a_w values in the end of ripening was not low enough in order to control the growth of most microbial groups analyzed. No significant variation in a_w was detected between season productions. Cheese moisture, however, significantly varied between the two periods. Therefore, the reduction was better evidenced in the samples ripened in Summer, due to high temperature and lower relative air humidity in the maturation room, which cause an extensive dehydration. Margolles *et al.* (20) analyzed the effect of climatic conditions of the different seasons on the features of some Spanish cheeses and observed that only cheese moisture varied significantly, with the higher values detected in winter.

The low concentration of NaCl at the watery phase during the ripening process was not enough to inhibit the growth of microbial groups.

The evolution that physicochemical parameters undergo with the ripening of *Serrano* cheese does not produce markedly antagonistic conditions for any of the microbial groups investigated. Therefore, the progressive reduction of the microorganisms in the core of the cheese is not a consequence of a single inhibitory effect, but rather the combination of several factors.

Although the microbiological countings and the main physicochemical characteristics of *Serrano* cheese have shown to vary significantly during ripening, the production itself did not present any important variations considering the season of the year, except for halotolerant counts, moulds and moisture content of cheeses.

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RESUMO

Evolução das características microbiológicas e físico-químicas durante a elaboração e maturação do queijo Serrano

As variações da microbiota de seis bateladas do queijo Serrano (3 maturadas no verão e 3 maturadas no inverno) foram estudadas durante a produção e maturação, com o objetivo de caracterizá-lo microbiologicamente. Este queijo é tradicionalmente elaborado com leite cru, sem a adição de cultura lática, por produtores rurais do sul do Brasil. Vários grupos microbianos foram enumerados no leite, na coalhada e em diferentes estágios ao longo dos 60 dias de maturação. Também foram investigados alguns parâmetros físico-químicos, tais como pH, acidez titulável, atividade de água, umidade e teor de NaCl. Determinaram-se os coeficientes de correlação entre os valores desses parâmetros e as contagens dos microrganismos ao longo da maturação. A influência da estação do ano também foi estudada. Os resultados demonstraram que a microbiota e as características físico-químicas do queijo Serrano apresentaram variações significativas durante o período de maturação de 60 dias, principalmente no verão. A maioria dos grupos microbianos atingiram suas contagens máximas no queijo aos 7 dias, diminuindo progressivamente até o final da maturação. O aumento nas contagens durante a primeira semana de maturação foi acompanhado por um declínio acentuado no valor do pH, devido à produção de ácido pelos microrganismos. As bactérias lácticas constituíram-se no principal grupo microbiano do queijo Serrano e suas contagens foram similares as de microrganismos viáveis totais em todos os pontos amostrados. O grande número de lactobacilos durante a elaboração e maturação do queijo sugere que estes microrganismos possam ter um papel importante na produção deste queijo. Em conclusão, o trabalho aponta a importância de um tempo de maturação superior a trinta dias como fator de estabilização microbiológica deste produto artesanal, bem como o fato de ser um produto bastante homogêneo quanto às suas características físico-químicas e microbiológicas, independente da estação do ano, exceto para as contagens de halotolerantes e de bolores e do teor de umidade, que apresentaram diferenças significativas entre o verão e o inverno.

Palavras-chave: queijo Serrano, maturação do queijo, microbiologia de alimentos, bactérias lácticas.

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