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The Effect of Temperature on Physicochemical and Microbiological Aspects of Serrano Artisanal Cheese Ripening

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HIGHLIGHTS

- SAC was free of staphylococcal toxin during ripening.
- *Staphylococcus* group did not reach legal parameters when cheeses were ripped at 5ºC.
- 20ºC and 12.5ºC for 30 days seems the ideal conditions to reach legal parameters.
- Main acid lactic bacteria were *Enterococcus* sp. and *Lactococcus* sp.

Abstract: The serrano artisanal cheese (SAC) is a traditional raw milk dairy produced in southern Brazil. The maturation temperature plays a crucial role regarding the cheeses' microbiological and physicochemical features and has been underexplored when it comes to unpasteurized milk dairy in Brazil. The objective of the present work is to evaluate the effect of ripening temperature on foodborne bacteria (total and thermotolerant coliforms, positive coagulase *Staphylococcus* and the production of its enterotoxin, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* spp.) and physicochemical attributes (moisture, water activity, pH, acidity and sodium chloride concentration) of SAC during maturation at 5ºC-20ºC for up to 60 days. Metagenomic analysis was also evaluated through the rDNA 16S method at ripening time of 0-60 days. The samples were safe for *L.monocytogenes, E.coli, Salmonella* spp. and staphylococcal toxin. The physicochemical parameters reached legal boundaries as follows: moisture, after 30 days at 5ºC; fat, after 30 days at 12.5ºC and 20ºC; fat at dry extract, after 30 days at 12.5ºC or 15 days at 5ºC. The microbiological features indicate that positive coagulase *Staphylococcus* reached legal conditions after 30 days when cheeses were ripped at 12.5ºC and 20ºC; thermotolerant coliforms after 30 days at 12.5ºC and 20ºC; and total coliforms after 15 days at 20ºC. Lower temperatures lead to lower inactivation rates, and *Staphylococcus* did not reach legal parameters at 5ºC positive coagulase regarding moisture, water activity

and pH reduction. Meanwhile, sodium chloride concentration and enhanced acidity played important role regarding foodborne pathogen inactivation. The main lactic acid bacteria within the cheeses during ripening were *Enterococcu*s sp. and *Lactococcus* sp.

Keywords: raw milk cheese; maturation; foodborne bacteria; acid lactic bacteria.

INTRODUCTION

Serrano artisanal cheeses (SAC) have been produced in the states of Rio Grande do Sul and Santa Catarina for almost 200 years from cattle's milk and fed by native pasture in small farms of the region [1], which gives the cheeses a unique organoleptic characteristic (color, aroma, flavor and appearance) [2]. SAC has been recently certified with an Origin Producing Label [3] and must be produced by traditional techniques within the territory located in Brazil's southernmost states, characterized by high altitude (between 400 and 1,400 meters above sea level) and severe winters. The SAC's production is an important tool for environmental preservation, as long as its milk comes from the cattle fed on native pastures of the region and slows down the advance of monoculture in the region, the utilization of pesticides, the reduction of vegetal and animal biodiversity, as opposed to changing the region's landscape [4]. In addition, its sales represent up to 50% of the properties' gross annual income, showing great social importance for small farmers [4]. Among the traditional production aspects, it is mandatory that SAC products are produced from unpasteurized milk, which has currently brought numerous discussions about food safety against traditional aspects [5].

Ripening is an important operation within the raw milk dairy products context, in order to control foodborne and spoilage microorganism growth. Biochemical and microbiological phenomena during cheese ripening impacts on flavors and texture, and it also helps to control the growth of spoilage and pathogenic microorganisms. Increasing the population of acid lactic bacteria, the production of organic acids and bacteriocins, beyond the competition for substrate with other microorganisms' impact on food safety [6]. The moisture's reduction during maturation decreases the cheese's water activity and increases salt concentration, acidity and other antimicrobial components that also contribute to dairy products' microbiological stabilization [7].

Nowadays, SAC is commercialized with 15 to 30 days of ripening, when the product is semi-hard and presents intense flavor and yellowish color [8]. However, current literature has indicated that it is possible to ripe unpasteurized milk cheeses for less than 60 days. Souza and coauthors [9] observed that serrano artisanal cheeses' ripening process should be longer than 30 days, but not necessarily 60 days, in order to allow microbiological stabilization. Pretto and coauthors [10] observed that ripening SAC for 33 days is enough to reach a safe foodborne microbial pattern. But there is still the need for food science and technology efforts to properly produce SAC products safely with a low ripening time.

Since SAC products are typically produced by small cheese makers characterized by small scale production, they present low parameters of production control [8-11], including ripening rooms' temperature. In the study of Souza and coauthors [9], experimental procedures were evaluated during the winter and summer periods, whereas Pretto and coauthors [10] did not control the maturation temperature and estimated to be approximately 10ºC. According to the current legislation, ripening must happen at a temperature above 5ºC [43], but in Rio Grande do Sul, the maturation is allowed to happen at room temperature [12]. In this context, SAC's producers still lack the knowledge to ensure proper maturation process, and temperature may be a critical parameter regarding the SAC's features. Therefore, this work aimed to carry out physicochemical and microbiological analyzes of the SAC during its maturation at three different storage temperatures (5ºC, 12.5ºC and 20ºC), to evaluate inactivated microorganisms, comparing them to the physicochemical analysis as well.

MATERIAL AND METHODS

Cheese production and ripening

SAC products were produced according to traditional standards pertaining to the serrano cheeses production [8, 13, 43] that take place in family agro-industry businesses located in São Francisco de Paula (RS, Brazil), which have been inspected and legalized in July, 2021. Raw milk was filtered and warmed to 32ºC for addition of commercial calf rennet (7 mL – to 10 L of milk). Coagulation happened at room temperature (approximately 20ºC) for 60 minutes until the right degree of consistency was achieved, when curd was removed and whey was drained. Curd was transferred into perforated plastic molds following a 24-hour whey drainage under pressure. The curds (average mass of 500 g) were then homogenized in a brine

solution (22% w/v sodium chloride) to mix the cheese mass with salt [43].

Ripening happened in controlled chambers (TE-371, Tecnal) for up to 60 days at temperature of 5ºC, 12.5ºC and 20ºC. The parameter of 5ºC was (adopted) because of legal crucial parameters and 20ºC is the average temperature during the whole year in Rio Grande do Sul, meanwhile 12.5ºC is the average temperature between 5ºC and 20ºC. During this maturation period, aliquots of QAS were collected every fifteen days to perform physicochemical and microbiological analyses.

Microbiological analysis

The *L. monocytogenes* population was evaluated by diluting 25 g of cheese in *Listeria* selective enrichment broth for 24 h at 30ºC, when aliquots were transferred to Fraser broth and kept for 48 h at 36ºC. Colonies from the Fraser system were transferred to Oxford agar, Palcam agar and Tryptose Nalidixic Acid agar and incubated at 36ºC for 72 h, whenever typical colony forming units (CFU) were observed. Results were expressed as presence or absence of *L. monocytogenes* in 25 g of product [45].

As for the*Samonella* spp. analysis, 25 g of cheese were diluted in 225 mL non-selective buffered peptone distilled water (ADPT) and incubated at 35°C for 24 h, when the culture was transferred to tubes with Rappaport broth and kept at 41°C for 24 h. Then, a selective-differential plating was performed in Salmonella-Shigella Agar and Bright Green Agar and plates and kept at 35°C for 24 h, whenever the presence of typical CFU was verified. Results were expressed as presence or absence of *Salmonella* in 25 g of product [46].

As for the thermotolerant coliform population count, 25 g of food were homogenized with 225 mL of 0.1% (w/v) peptone water and further decimal dilutions added to Red Violet Bile Agar at 36ºC for 24 h. Typical colony forming units (CFU) were selected and transferred to Duhram tubes with EC broth and incubated at 45ºC for 48 h whenever the presence of gas formation was verified. Results were expressed as CFU of thermotolerant coliforms per gram of cheese [47].

As for the positive coagulase *S. aureus* analysis, aliquots of serial dilutions of the dairy product in 0.1% (w/v) peptone water were added to Baird-Parker agar and incubated at 36ºC for 48 h whenever typical CFU were verified. As for the positive coagulase analysis, typical CFU were transferred to Brain Heart Infusion broth for 24 h at 36ºC whenever aliquots were added to rabbit plasma and coagulation was observed. Results were expressed as CFU of positive coagulase *S. aureus* per gram of cheese [48]. The microbiological parameters for the QAS followed the Brazilian legislation for cheeses [26].

Physicochemical analysis

Moisture was evaluated in a static air oven at 105°C until constant weight [49]. The dry extract was established by the relationship between the fat content and the total dry extract of the cheese [21]. The pH was measured using the pH meter equipment (Datalogger-Digital Instruments model). Titratable acidity was measured using a standardized 0.1 M sodium hydroxide solution [15]. Water activity was measured at 20ºC in the LabSwift equipment [14]. The argentometric method was used to establish the percentage of sodium chloride in the cheese samples. This method is based on the titration of chlorides against silver nitrate, precipitating as silver chloride at a slightly alkaline pH, according to Brazilian standard procedure [15]. The physical-chemical parameters for the QAS followed the current regulations [43, 44].

Analysis of lactic acid bacteria by metagenomic analysis

Samples of 250 g of cheese collected at five different points of the cheese were analyzed; from the manufacturing day and within sixty days of maturation (at 5, 12.5 and 20ºC). The identification of lactic acid bacteria was performed using a high-performance sequencing of the v3/v4 regions of the 16 s rRNA gene. The libraries were prepared according to a protocol from Neoprospecta Microbiome Technologies, and amplification was performed with primers for the v3-v4 region of the 16S rDNA gene, 341f (CCTACGGGRSGCAGCAG) [16] and 806r (GGACTACHVGGGTWTCTAAT) [17]. The libraries were sequenced by using the MiSeq Sequencing System equipment (Illumina Inc., USA), and the sequences were analyzed by using the Sentinel pipeline.

Taxonomic identifications were performed with blastn v.2.6.0+ [18], by using a database from the company Neoprospecta® as a reference. In order to identify the species of microorganisms, present in the samples, the DNA sequences obtained were compared with a database containing other DNA sequences from already characterized species. The sequencing was performed on the MiSeq Sequencing System (Illumina) equipment.

Data analysis

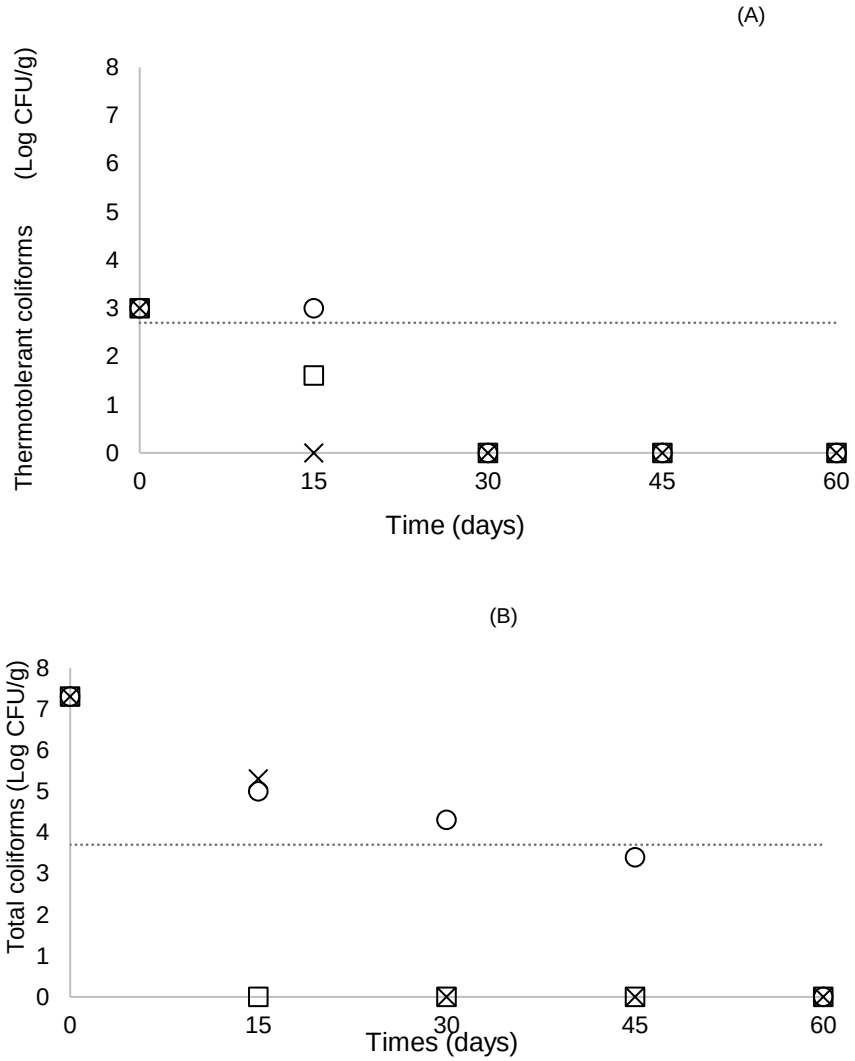
The experiments were conducted in triplicates and average data was compared by Student t-tests and differences were considered significant when $p<0.05$.

The correlation between bacteria population and physicochemical parameters was evaluated by Pearson's correlation values. Moreover, Partial Least Square (PLS) regression, a multivariate method that establishes linear relationships between a set of predictors (X-block, physicochemical parameters) and responses (Y-block, microorganism population), was performed to each group of bacteria in order to establish which physicochemical parameters are mainly related to the microorganism inactivation.

All statistical analyses were performed using XLSAT [50].

RESULTS AND DISCUSSION

Population reduction of foodborne bacteria during ripening of serrano cheese was evaluated and results showed that dairy products are free of *Salmonella* spp., *E. coli* and *L. monocytogenes* through the maturation period. Pretto and coauthors [10] also observed the absence of *Salmonella* spp. and *L. monocytogenes* on SAC, although is not rare to find these bacteria on artisanal dairy products [19-20]. Brazilian regulation establishes their absence in cheeses whose analysis are performed in the end of maturation period [21]. Thermotolerant and total coliforms and positive coagulase *Staphylococcus* population behavior through ripening for 60 days at 5ºC, 12.5ºC and 20ºC are shown in Figure 1



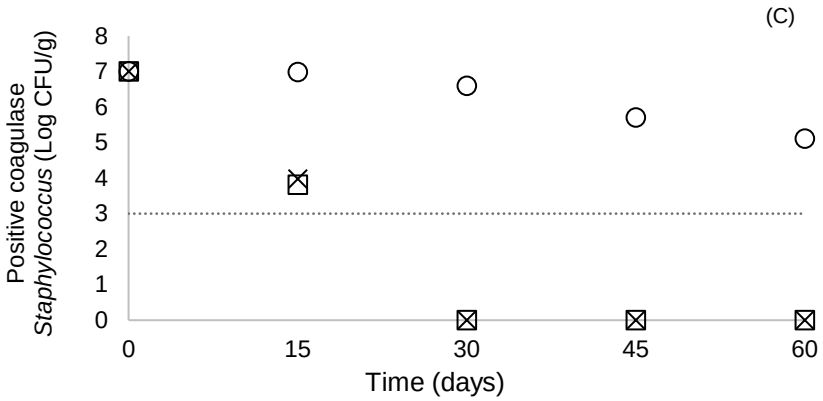


Figure 1. Thermotolerant coliforms (A), total coliforms (B) and positive coagulase *Staphylococcus* (C) population behavior during serrano artisanal cheese ripening at 5ºC (o), 12.5ºC (□) and 20ºC (x). The dashed line represents maximum limits according to Brazilian regulation for the parameter analyzed. *E. coli* and *L. monocytogenes* populations were not detected during the ripening period.

Initial thermotolerant coliform counts in SAC were 3.0±0.0 log CFU/g. Higher counts (4.27 log CFU/g) were observed by Souza and coauthors [9] and Pretto and coauthors [10] (6.98 log CFU/g) for SAC, indicating inadequate hygienic production conditions of the cheeses throughout the current work [44]. Figure 1A shows that the bacteria population decrease presented an exponential decay during the ripening period, which behavior has been reported previously by Pretto and coauthors [10]. At 5ºC, bacteria inactivation presents an initial shoulder behavior, indicating resistance to inactivation at the beginning of the ripening process. At 20ºC the population counts after 15 days of maturation were 1.6±0.0 log CFU/g, which is below the regulation threshold for proper commercialization, whereas the thermotolerant coliform population was below the detection threshold (<10 colonies NMP/g) whenever ripening happened at 12.5ºC before the 15-days ripening period. Souza and coauthors [9] observed that mild to low temperatures in maturation room during SAC production did not impact the reduction of these microorganisms groups, whereas warm temperatures did help on inactivation.

Total coliforms in SAC products analyzed at the beginning of maturation were 7.0±0.0 log CFU/g and decayed exponentially over time (Figure 1B), furthermore, non-initial resistance was observed. After 15 days when ripening happened at 20ºC, the population of total coliforms was already below the detection limit, indicating a very fast inactivation, whereas at 12.5ºC, this phenomenon happened after 30 days. However, when SAC was left to ripe at 5ºC, the bacteria population was found below $5x10^2$ CFU/g (the Brazilian regulation limit [21]) just after 45 days, and it was bellow detection limit after 60 days. Souza and coauthors [9] also observed that the rain that occurs during the winter had less impact on the inactivation of total coliforms in SAC products than during the summer, due to the higher temperatures in the region in this period, thus, fortunately, for a rapid decrease of these microorganisms, since the cheese temperature does not occur under temperature controlled environment. Similar results were observed to produce Monchego cheeses: when the room ripening temperature was enhanced from 10ºC to 20ºC, the total coliforms reduction rates were highly enhanced [22].

Figure 1C shows the inactivation behavior of positive coagulase *Staphylococcus*. Initial counts were 7.0 log CFU/g and quickly decayed at 12.5ºC and 20ºC, until they reached the detection limit between 15-30 days. However, the population reduction was slower and did not reach the regulation limit (10^3 CFU/g) until 60 days of ripening [26] whenever maturation happened at 5ºC. The presence of coagulase-positive *Staphylococcus* during SAC maturation at 5ºC may be caused by lower reduction of pH by lactic acid bacteria which is an important phenomenon for inhibition of bacteria from *Staphylococcus* genus in cheeses [23]. Pretto and coauthors [10] observed that SAC products ripped at 10ºC, took 30 days to decrease initial population of about 7 log CFU/g to below 3 log CFU/g. The *Staphylococcus* genus is divided according to the synthesis or non-synthesis of the enzyme coagulase, and its production is correlated to pathogenicity that causes toxin-poisoning [24]. They are an enterotoxin-producing bacteria and literature indicated that population above 5 log UFC/g may produce the toxin in foods [25]. Current Brazilian Regulation [26] indicated absence of staphylococcal enterotoxin in cheeses for commercialization. Results of the present work showed the absence of staphylococcal toxin in SAC during maturation in the temperature range of 5ºC-20ºC, although the initial population of bacteria was quite high. The production of toxin by Staphylococcus spp. depends on pH (near the neutral), high Aw (above 0.99), temperature (optimal production at 37ºC) and the presence of other microorganisms [27], moreover, the production of enterotoxin has been reported to begin with

detectable amounts after 24 h of incubation at 30ºC [28]. Thus, the results indicate important food safety conditions of SAC products regarding this toxin, a trend topic currently in Brazil concerning raw milk cheeses.

Brazilian regulation establishes some standards for physicochemical parameters. Moisture content is not a direct parameter for microbiological evaluation, but it is related to water activity with absorption isotherms, according to Figure 2.

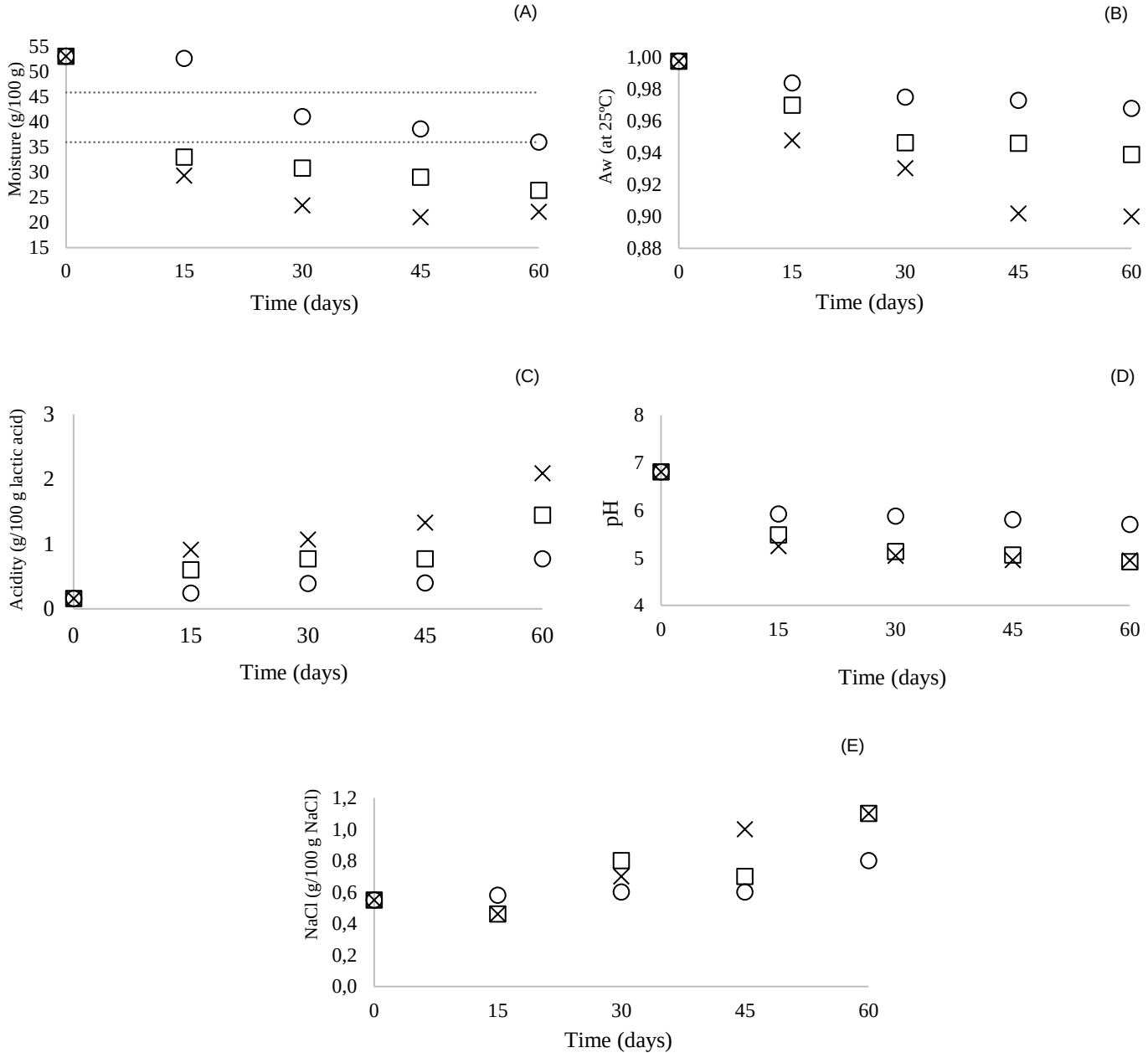


Figure 2. Moisture (A), water activity (B), acidity (C), pH (D) and sodium chloride concentration (E) changes during serrano artisanal cheese ripening at 5ºC (o), 12.5ºC (□) and 20ºC (x). The dashed line represents minimum and maximum limits according to Brazilian regulation for the parameter analyzed.

Figure 2A shows the SAC's moisture content during ripening and an exponential decay over time was observed as expected due to a drying behavior. The increase of ripening temperature implied on significant ($p<0.05$) reduction of water content in the samples and on faster water removal from SAC. At the end of the incubation period, at 5ºC, moisture content was found to be 36±0.02% and at 12.5ºC, 26.4±0.0%, whereas at 20ºC it was 22.1±0.03%. The increasing temperature implies higher water molecules kinetic energy, which facilitates water evaporation; whereas lower temperatures may present lower air relative humidity, which contributes to faster drying and lower moisture balance [29]. Brazilian regulation establishes a minimum moisture content of 36% and a maximum of 45%, whose limit was reached at 5ºC between 30-60 days; and

at 12.5ºC and 20ºC after 15 days, the SAC's moisture content was below the minimum limit (36%). Food dehydration depends on intrinsic and extrinsic features, thus, different cheese's shape and weight may change drying behavior. SAC products are reportedly produced in cylindrical and cobblestone shapes, in addition to weighing from 0.5kg to 1.5kg [8-11], being heavier and having a higher contact surface prone to lose moisture more slowly [29, 30, 31].

SAC's Aw reduced linearly (R^2>0.80) during maturation and at the end of the maturation period at 5ºC Aw was 0.968±0.0, at 12.5ºC 0.939±0.0 and at 20ºC, 0.900±0.0 (Figure 2B). Foodborne and spoilage bacteria present growth limitation in foods with Aw lower than 0.90, whereas spoilage mold and yeast was 0.8 [32]. The results indicate that cheeses ripped for 60 days at 5ºC and 12.5ºC still present high values of Aw, which allows for bacteria and fungi growth, thus, changes on the product might still happen during the products' shelf-life. According to Hoffmann [33], bacteria are generally more demanding regarding the availability of free water in the food, and most of them grow in a minimum water activity of 0.91 – 0.88.

The pH decrease and acidity enhancement in SAC during ripening is demonstrated in Figure 2D and 2C, which is caused by LAB growth and moisture reduction, and consequently concentration of LAB's metabolites, mainly organic acids. A high reduction ($p<0.05$) of pH was found in the first 15 days of ripening and then reduced from 6.8±0.0 to 5.7±0.0, 4.9±0.0 and 4.9±0.0 when ripening happened at 5ºC, 12.5ºC and 20ºC, respectively, at 60 days of maturation. Souza and coauthors [9] observed increase on pH values of SAC during the first and the fourth ripening week during summer season as the result of the metabolic activity of mold and yeast, which utilize lactic acid as a source of carbon, and/or the proteolytic process that releases great amounts of nitrogenous alkaline compound. Whereas the acidity linearly enhanced in all the temperatures studied from 0.2±0.0 g/100g at the beginning of the process and reached 0.8±0.0 g/100g, 1.4±0.0 g/100g and 2.1±0.0 g/100g at 5ºC, 12.5ºC and 20ºC, respectively.

In addition to fermentative functions, the enterococcus genus produce enterotoxins, which are part of bacteriocins that help inhibit certain types of pathogens (such as *Staphylococcus spp*., *Clostridium spp*., *Bacillus spp.* and *Listeria spp*.) [34], during cheese fermentation and ripening [35], however, this group is considered to be a disease-causing group in hospital settings because they have increasing antimicrobial resistance [36]. The *Lactococcus* genus of LAB is widely utilized in the production of cheeses and milk curd, because it has the function of developing acidifying and proteolytic activities, and, consequently, texture, flavor and aroma to cheeses, besides acting as biopreservatives, through the production of antimicrobial compounds, such as organic acids and bacteriocins [37].

NaCl concentration in SAC behavior is presented in Figure 2E, which increased from 0.55±0.0 g/100g to 0.80±0.0 g/100g, 1.10±0.0 g/100g and 1.10±0.0 g/100g, when maturation happened at 5ºC, 12.5ºC and 20ºC, respectively, mainly after 15 days of ripening.

Results show that bacteria inactivation depends on the temperature of SAC exposure, but also indicate that physicochemical parameters play an important role. Table 1 shows Pearson's correlation *r*-values between thermotolerant coliforms, total coliforms and positive coagulase *Staphylococcus* and physicochemical parameters during ripening for all experimental data. Results show positive relation between moisture, Aw and pH and bacteria growth and negative relation with NaCl concentration and acidity. Thermotolerant coliform population was significantly impacted ($p<0.05$) by moisture and pH, whereas total coliforms and positive coagulase *Staphylococcus* were impacted by moisture and pH ($p<0.05$). Aw and NaCl concentration did not have a significant impact ($p>0.05$) on the pathogenic bacteria.

Table 1. *r*-values of correlation between foodborne bacteria in SAC and cheese's physicochemical parameters during ripening.

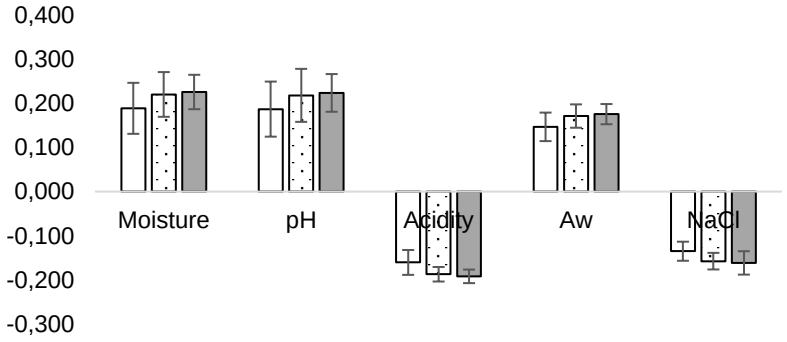
Pathogenic Microorganisms	Moisture	Aw	pH	Acidity	NaCl
Thermotolerant coliforms	0.84*	0.58	0.81*	-0.62	-0.52
Total coliforms	0.89*	0.72	0.91*	-0.79*	-0.64
Positive coagulase *Staphylococcus*	0.91*	0.74	0.89*	-0.83*	-0.71

* Statistically significant correlation ($p<0.05$).

Souza and coauthors [9] observed that microorganism's reduction in SAC is a combination of several factors, and no individual physical-chemical feature had antagonistic conditions for this decrease. However, evaluation of the impact of different parameters on complex food matrices' characteristics by conventional "one-at-a-time-approach" and conventional statistical evaluation may lead to a critical analysis and disregards the importance of interaction of process parameters. Multivariate analysis is particularly suited to

solve analytical problems in the food industry, when the effects of processing variables on a complex product are involved [38]. Figure 3 shows the PLS regression results and contributes to discuss the main parameters that have impact on bacteria counts. Positive values indicate that increasing the parameter positively influences the bacteria population growth and negative values imply that enhancing the physicochemical parameter value influences their inactivation. The columns' size represents the attribute's influence on a pathogenic bacteria group, which could be positive or negative. The vertical lines represent an interval of 95% confidence crossing the x-axis, and the corresponding parameter does not have influence on the microorganism growth. In order to obtain a successful regression model, R^2X and R^2Y had to be equal or superior to 0.70. The ability to predict new samples was evaluated by Q^2, that should be equal or superior to 0.50 [39]. Table 2 shows the statistical fitting parameters for the regression model made from the PLS technique, which presented good adequacy to the experimental data. Global analysis of the three bacteria group (positive coagulase *Staphylococcus*, thermotolerant and total coliforms) showed Q^2-value of 0.68 and R^2Y and R^2X values of 0.71 and 0.83, respectively. The impact of moisture, pH, acidity, Aw and NaCl concentration are represented by standardized coefficients shown in Figure 3. Positive standard coefficient values indicate positive relation between the results, which means that higher values of moisture and pH lead to a higher bacteria population. Physicochemical parameters presented similar effect on the three bacteria, although moisture and pH demonstrated to be slightly more important than the others. Standardized coefficients of moisture were in the range of 0.19-0.23; of pH in the range of 0.19-0.22; for acidity ranged between -0.16 and -0.19; of Aw ranged from 0.15 to 0.18; and for NaCl concentration between -0.14 and -018. Therefore, results show that the inactivation of the microorganisms in SAC is not caused by a single inhibitory effect, rather, it's caused by the combination of several factors.

Figure 3. PLS results for influence of physicochemical parameters on Thermotolerant coliforms (white bars), total coliforms (dotted bars) and positive coagulase *Staphylococcus* (grey bars) population growth.



The LAB identified in the cheese samples belong to the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales* and were divided into five families (*Enterococcaceae, Lactobacillaceae, Streptococcaceae, Leuconostocaceae* and *Streptococcaceae*) and five genera (*Enterococcus, Lactobacillus, Lactococcus, Leuconostoc* and *Streptococcus*). Seventeen species were identified in the bacterial microbiota of cheeses (*Lactobacillus brevis, Leuconostoc pseudomesenteroides* (5 reads, respectively); *Lactobacillus amylovorus* (7 reads); *Enterococcus faecium* and *Enterococcus thailandicus* (12 reads, respectively); *Enterococcus pseudoavium* (22 reads); *Enterococcus durans* (59 reads); *Enterococcus devriesei* (70 reads); *Enterococcus casseliflavus* (84 reads); *Streptococcus dysgalactiae* (114 reads); *Lactococcus lactis* (236 reads); *Leuconostoc mesenteroides* (475 reads); *Lactobacillus casei* (649 reads); with 120.953 reads of species of the genus *Lactococcus* sp. and *Enterococcus* sp. (163.755 reads) could not be classified at the species level of species found. Souza and coauthors observed that *Lactobacilli* were the most abundant lactic bacteria, followed by *Enterococci* and *Lactococci* in SAC. Souza and coauthors [9] reported that SAC presents LAB populations from the following genera: *Lactobacillus, Lactococcus, Enterococcus*, and *Leuconostoc,* which change throughout their production and maturation. Delamare and coauthors [40] observed that SAC from Rio Grande do Sul presented 278 of LAB, with the most abundant *Lactobacillus* genus, having an abundance 91%, followed by *Lactococcus* (7%) and *Enterococcus* (2%), according to Table 2.

Table 2. Lactic acid bacteria (LAB) species identified throughout the maturation of the QAS (0-60 days).

LAB	Ripening temperature					
	5ºC		12.5ºC		20ºC	
	Day 0	Day 60	Day 0	Day 60	Day 0	Day 60
Enterococcus casseliflavus	0	0	0	0	0	1.33
Enterococcus devriesei	0.03	0	0.03	0.03	0.03	0.03
Enterococcus durans	0.04	0.12	0.04	0	0.04	0.02
Enterococcus faecium	0.01	0.01	0.01	0	0.01	0
Enterococcus pseudoavium	0	0.02	0	0	0	0
Enterococcus sp.	50.6	14.5	50.6	94	50.6	45.38
Lactobacillus amylovorus	0	0	0	0.07	0	0.02
Lactobacillus casei	0	0.02	0	0.43	0	0.18
Lactococcus lactis	0.1	0.15	0.1	0.01	0,1	0.11
Lactococcus sp.	48.99	84.88	48.99	5.1	48.99	52.73
Streptococcus dysgalactiae	0.22	0.1	0.22	0.36	0.22	0.2
Leuconostoc mesenteroides	0.02	0.19	0.02	0	0.02	0

Table 2 shows that the predominance of bacterial genera in SAC were *Lactococcus* sp. and *Enterococcus* sp. obtained an abundance of 48.99% and 50.6%, respectively, on the day of their manufacture. On the last day of SAC maturation there was a predominance of the genus *Lactococcus* sp. (84.88%) at a temperature of 5ºC, and a decrease of the genus *Enterococcus* sp. (14.5%). At the maturation temperature of 12.5ºC, there was a greater predominance of the genus *Enterococcus* sp. (94%), as opposed to *Lactococcus* sp. (5.1%). In the SAC matured at 20ºC, there was a small difference between these species if compared to the first day of cheese making; the *Lactococcus* had (52.73%), and the *Enterococcus* sp. (45.38%). It has been shown that 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. According to Peterson and coauthors [41] the formation of several metabolites, such as lactate, citratre, glycerol, and amino acids, among others, which are better utilized by lactobacilli, takes place during the fermentative process of cheese. According to Foulquie Moreno and coauthors [42] a greater abundance of enterococcus during the final stage of cheese maturation may occur due to its tolerance to a wide range of environmental conditions.

CONCLUSION

In conclusion, results showed that the temperature of SAC ripping plays important role on the dairy's microbiological and physicochemical features and it is crucial that's controlled during the product manufacturing. Samples were safe of *L. monocytogenes, E. coli, Salmonella* spp. and staphylococcal toxin within the maturation period, indicating important food safety aspects of SAC.

A higher temperature of ripening (12.5 and 20ºC) for 30 days, showed to have the most proper conditions in the conditions studied, as the foodborne bacteria reached a safe population. Maturation of SAC at lower temperatures (5ºC) showed to be inadequate, as coagulase positive Staphylococcus microorganisms were not inactivated throughout the incubation period (60 days), indicating the need to renewthe current Brazilian regulation concerning raw milk cheeses.

The lactic acid bacteria species predominant in the SAC's experiment were the genera *Lactococcus sp*. and *Enterococcus sp*. These endogenous microorganisms also contribute to the inactivation of pathogenic microorganisms.

Statistical results show that the inactivation of the studied microorganisms depends on several physical-chemical factors, such as Aw, titratable acidity and NaCl content. Therefore, results of the current work support the possibility of ripening SAC products for commercialization in less than sixty days, as long as the maturation temperature is controlled.

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