Heliyon 6 (2020) e04446

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Artisan minas cheese of Serro: proteolysis during ripening

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ARTICLE INFO

Keywords:
Food science
Electrophoresis
Ripening extension index
Ripening depth index
Firmness
Moisture

ABSTRACT

The Artisan Minas Cheese (AMC) is the oldest and most traditional Brazilian cheese, it is produced in several regions of the state of Minas Gerais, such as the Serro region. The most striking features of the AMC-Serro are the use of raw milk and natural bacteria from the whey, popularly known as *pingo*, as well as the use of the rind washing process. The aim of the present study was to evaluate the proteolysis of the AMC-Serro from three different producers, during 60 days of maturation, and to relate the proteolysis to the producing farms, the production season and the rind washing during ripening. For this purpose, TRICINE-SDS-PAGE, proteolysis extension and depth indices, moisture, and texture (firmness) were evaluated. It was concluded that the temperature and moisture of the cheeses, that were determined by the location of the ripening room, the production season and the rind washing, were the most important factors. The degree of proteolysis also had an impact on the water loss during ripening, with effect on cheese safety. The results obtained in this study may be used to better understand the transformations during ripening of AMC-Serro and help the small traditional farmers to improve their product's quality and stability.

1. Introduction

Artisanal cheeses show unique characteristics, which vary according to the region and the milk microbiota where they are produced and contribute to their inherent social and cultural value (Santilli, 2015). The Artisan Minas Cheese (AMC) is the oldest and most traditional Brazilian cheese and is produced in several regions of the state of Minas Gerais. Among them is the Serro region, that encompasses a large area (2,258 km^2) including small villages with 3,600 inhabitants and larger towns with up to 18,000 residents. The cheesemaking culture in the Serro region is traditional for Brazilian standards, with almost 300 years of history, and the know-how for the local artisanal cheese production has been passed down from generation to generation over the years (Monteiro, 2018).

The most important features of the AMC-Serro are the use of raw milk and of natural starter cultures of the whey from the previous cheese production, popularly known as *pingo* – which loosely translates as "drop" (Perin et al., 2017). The *pingo* contains a high concentration of sodium chloride and many endogenous lactic acid bacteria, responsible for the typical sensory characteristics of the cheese of each producer (Bachmann et al., 2011). Other peculiarities are the short maturation period of at least 17 days and the use of the rind washing process, that occurs every two or three days depending on the environmental conditions of temperature and humidity of the maturation room, and consists of rinsing the surface of the cheeses with water (Chaves et al., 2018). However, some producers are innovating and eliminating the washing step, which allows fungi to grow on the rind. The AMC-Serro is recognized by the Institute of National Historical and Artistic Heritage (IPHAN - "Instituto do Patrimônio Histórico e Artístico Nacional") as an intangible heritage in Brazil, and was the first Brazilian cheese to receive the Geographical Indication from the National Institute of Intellectual Property (INPI - "Instituto Nacional de Propriedade Intelectual") in 2011 (IPHAN, 2008; Monteiro, 2018), nevertheless, there are only a few studies published concerning its production and quality to date.

Over the course of ripening, cheeses loose water and undergo a series of changes that result in sensory, chemical and biochemical modifications, transforming the final product (McSweeney, 2004; Salum et al., 2018). The intensity of proteolysis is highly variable and depends, among other factors, on the enzymes in the rennet, the composition of the endogenous milk microbiota and the added microorganisms, the endogenous proteases in the raw milk and on those produced by the

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https://doi.org/10.1016/j.heliyon.2020.e04446
Received 18 March 2020; Received in revised form 20 May 2020; Accepted 9 July 2020
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different microorganisms present (Cavalcante et al., 2007; Fox et al., 2015). To monitor the cheese ripening process, it is useful to chemically determine the extension and depth of proteolysis, that help to quantify and characterize the contribution of the many agents acting on the cheese proteolysis during ripening (McSweeney, 2004). Although the study of proteolysis is of great importance to understand the cheese maturation process, little information is available on the proteolysis of the AMC in general and of the AMC-Serro in particular (Costa Junior et al., 2009; Pinto et al., 2017; Silva et al., 2011; Sobral et al., 2015). Due to the absence of data on proteolysis of Brazilian artisanal cheeses, the present study aimed to evaluate the proteolysis of the AMC-Serro during 60 days of ripening, and to relate these data to the producing farms, the season of production and the application of the rind washing process during ripening.

2. Materials and methods

2.1. Sampling

For this study, samples of cheese were collected from three different farms/producers (P1, P2 and P3). In each producing farm, ripening occurred in a ripening room with a particular micro-climate (temperature and relative humidity) determined by its location. The ripening room in P1 was in an area with little exposure to direct sun, with an average temperature of 18,2 °C and 68% relative humidity in winter, and 21 °C and 78% in summer. The ripening room in P2 was in the shade, under the canopy of a tree, with an average temperature of 17 °C and 72% relative humidity in winter, 20 °C and 81% in summer. And in P3 the ripening room was exposed, with direct incidence of the afternoon sun with an average temperature of 18.5 °C and relative humidity of 67% in winter and 22.5 °C and 78% in summer. The temperature and relative humidity data were recorded using a thermo-hygrometer (Instrutherm Mod. HT-70, São Paulo, BR) according to the manufacturer's instructions. The measuring instrument was placed on a shelf of cheese, in the maturation room, at a height of 1.8 m. The maturation rooms, with an area of approximately 20 m^2, were made of brick, lined with ceramic and contained wooden shelves on which the cheeses were ripening.

All cheeses were produced with raw milk, following the traditional processing techniques of the Serro region: the *pingo* (the salted whey collected on the second day after coagulation) was used as inoculum, and industrial rennet (Ha-La®, Chr. Hansen, microbial chymosin, *Aspergillus niger* var. *awamori*) was added to the milk. P1 and P2 did not apply the periodic rind washing process on the surface of the cheeses, but cheeses from P3 were rinsed every two or three days along the ripening period.

The cheeses were matured in the original farms and had approximately 1 kg, the samples were collected after 3, 17 and 60 days of ripening (3D, 17D and 60D). The AMC-Serro samples were collected in two seasons, during the winter of 2017 and during the summer of 2018. All the results showed in this study represent the mean of three batches of cheese processing in each farm.

The samples were frozen at -18 °C to interrupt the ripening process and transported to the Embrapa Food Agroindustry facility, in Rio de Janeiro, where the samples were prepared and analyzed.

2.2. Preparation of extracts

The cheese samples were ground in a domestic food-processor (Mixer Philips Walita Viva Collection, Barueri, BR) for two minutes and then frozen and freeze-dried for 24 h (LioTop, model L101, São Carlos, BR). The extracts preparation and the TRICINE-SDS-PAGE procedure followed the methodology described by Carneiro et al. (2019).

2.3. TRICINE-SDS-PAGE

The analyses of caseins and possible products generated by proteolysis during ripening were performed in a modified TRICINE-SDS-PAGE system using the gel preparation technique according to Schägger and Jagow (1987). The gel run was performed on a Biorad® brand Power-pack Basic (Hercules, USA) equipment. Three different acrylamide gels were used at the following concentrations: 16.5% for the separation gel; 10% for spacing gel and 4% for sample application gel. Thirty microliters of the previously degreased samples were applied, the run was started under 15 V for 15 h and continued for 6 h in an 85 mA current.

A Bio-Rad® peptide standard containing the following proteins was used: 26,625 kDa triose phosphate isomerase; myoglobin, 16,950 kDa; α-lactalbumin, 14,437 kDa; aprotinin, 6,512 kDa; β-oxidized insulin, 3,496 kDa and bacitracin, 1,423 kDa.

After the end of the run, the gel was placed in a fixative solution with 50% methanol and 10% acetic acid under stirring for one hour. Subsequently, the gel was washed in distilled water and immersed in a dye solution containing 0.025% Coomassie G250 blue in 10% acetic acid for 2 h. For bleaching the gel, a 10% acetic acid bleach solution was used, the gel was immersed under stirring for 2 h and the solution was changed every 30 min. At the end of this step, the gel was washed in distilled water and then scanned (Image Scanner III model GE Scanner®) for image record.

2.4. Proteolysis extension and depth indices

To assess the extent of proteolysis, the total nitrogen (TN), the water soluble nitrogen at pH 4.6 (WSN), the non-protein nitrogen (NPN), and soluble nitrogen in 12% (v/v) trichloracetic acid (TCASN) were determined by the Kjeldahl method (according to the IDF methodoly, 1993). The total protein (TP) content was determined indirectly by multiplying the percentage of TN by the factor of 6.38, indicated for protein derived from milk (IDF, 1993). The ripening extension index (Eq. (1)) and the ripening depth index (Eq. (2)) were determined according to Pereira et al. (2008) and Pereira et al. (2010).

$$Ripening\ \ extension\ \ index(\%) = \frac{(WSN\ at\ pH\ 4.6 \times 100)}{TN} \quad (1)$$

where WSN means water soluble nitrogen and TN means total nitrogen.

$$Ripening\ \ depth\ \ index(\%) = \frac{(TCASN \times 100)}{TN} \quad (2)$$

where TCASN means trichloroacetic acid-soluble nitrogen and TN means total nitrogen.

2.5. Moisture and firmness

Moisture was determined in triplicate following the method recommended by AOAC International 930.15 (2010).

The firmness of the cheese samples was determined using cylindrical samples with 20 mm diameter and 20 mm height, taken from the central region of each sampled cheese. The measurements were performed using a texturometer (TA.XT2 Plus® Stable Micro Systems Texturometer Stable Micro Systems®, Haslemere, UK) equipped with a wire probe. The results were obtained and analyzed using Exponent Lite version 5.1® software (Stable Micro Systems). The equipment was calibrated with a standard weight of 5 kg. In the pre-test, the device's descent and the shear rate were 200 mm/min and in the test and in the posttest the speed was 2 mm/s and the samples penetration distance was 15 mm (Marinho et al., 2015). Analyzes were performed in triplicate.

2.6. Statistical analysis

The statistical analysis of the data was performed using the GraphPad Prism (5.0) software (GraphPad Software® Inc. San Diego, CA, USA) applying 2-way ANOVA with the Bonferroni posttest and $p < 0.05$ was considered significant.

3. Results

3.1. TRICINE-SDS-PAGE

The SDS-PAGE results obtained presented well-defined and clear casein bands, with different hydrolysis levels throughout the ripening period, evidencing the influence of several factors. The removal of fat from the extract provided adequate runs, resulting in gels without spots, with well-marked bands, allowing their identification. Figure 1 shows the electrophoresis gel of the cheese samples after 3, 17 and 60 days of ripening, in winter and summer, for P1, P2 and P3.

Figure 1A and B show the results for samples obtained from P1. Well-marked bands were observed at the beginning of the ripening, showing the preservation of the casein fractions (α and β). From the 17th day of ripening, there was a decrease in the intensity of these bands and the appearance of more pronounced bands of lower molecular weight, evidencing the occurrence of proteolysis. After 60 days of ripening, the bands of smaller molecular weight became even more intense. Comparing both gels (A and B) in Figure 1, the samples showed similar behavior during the winter and the summer.

In Figure 1C and D the gel images show the samples obtained from P2. The presence of well-marked bands of the different casein fractions can be observed. No noteworthy change was observed until the 17th day of ripening, indicating that truly little proteolysis occurred during both seasons summer and winter. After 60 days, the intact casein bands presented lower intensity and there was the appearance of lower molecular weight bands, showing the occurrence of proteolysis, which was much more intense in summer (1D) than in winter (1C). This may be observed

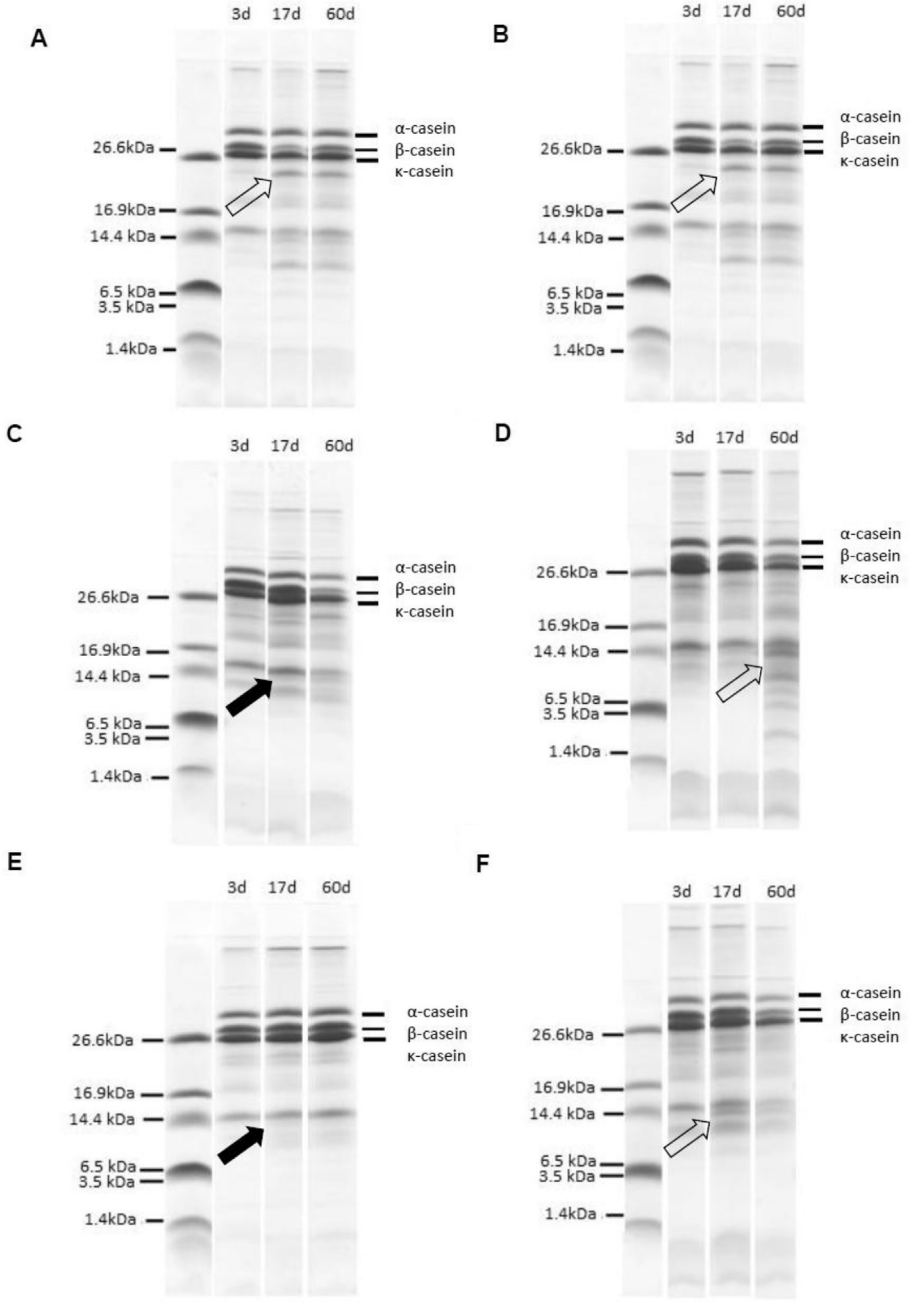

Figure 1. Electrophoresis gels: (A) P1 Winter 2017; (B) P1 Summer 2018; (C) P2 Winter 2017; (D) P2 Summer 2018; (E) P3 Winter 2017; (F) P3 Summer 2018. Hollow arrows indicate where proteolysis occurred and full arrows indicate where proteolysis did not occur. For original non-adjusted gels see Supplementary Figures 1–4.

by the high decrease of intensity of the caseins bands and by the higher number and intensity of lower weight bands after 60 days, in Figure 1D (summer) but not in Figure 1C (winter). It is noteworthy that a very efficient hydrolysis could lead to the formation of peptides with molecular weight below the detection limit of the method used, which was of 1 kDa.

In Figure 1E and F are the gels from P3 and no visible proteolysis during the maturation time may be observed, especially in the winter (1E). The ripening during winter (Figure 1E) showed almost no casein hydrolysis, as the proteolytic profile of the samples remained remarkably similar during the 60 days studied. Only in summer, there was some proteolysis, the bands of the casein fractions became less intense and bands of smaller molecular weight appeared. These samples behaved very differently from those obtained from P1 and P2.

3.2. Proteolysis and ripening extension and depth indices

The results obtained for the ripening extension and depth indices of proteolysis were presented in Figure 2A and B, respectively. Producer and season seemed to be the most important variables for both indices, whereas time seems to show influence only regarding the season. The cheese samples from P1 and P3 showed similar behavior: no significant alteration in the ripening indices during the winter and significant increase in both, extension and depth indices, during the summer. The samples from P2 behaved differently, with a slight but significant increase in ripening extension index during the winter, but no other significant alterations. The highest values of ripening extension and depth indices were found for the samples from P1, after 60 days of ripening, in summer. Regardless of the initial behavior, in the summer, the samples from P2 and P3 did not show significant difference at the end of the maturation time. On the other hand, in winter, the cheeses from P1 and P2 showed similar results after 60 days, that were significantly different from the lower values achieved by the cheeses from P3.

3.3. Moisture and firmness

The data for moisture content and instrumental firmness were shown in Figure 3A and B, respectively. All samples showed a significant moisture decrease over time, except for the cheeses from P3, that showed no significant reduction in moisture content throughout the ripening period, during the summer. The cheeses from P3 showed the highest moisture loss, after 60 days, in winter (there was a 50% reduction) and the lowest in the summer (34%), whereas cheeses from P1 and P2, both in winter and summer, presented the same decrease in moisture content of around 40%. The moisture content of the samples from all the producers were significantly higher in winter than in summer, and there was no difference between the moisture of the different producers in the latter season, while samples from P3 showed the lowest moisture content during winter.

The firmness observed for the samples from P1 and P3 increased significantly during ripening, in both seasons, but the samples from P2 showed no significant difference in firmness during maturation, regardless of the season. The highest increase in firmness were recorded for the cheeses from P3 during winter (370%) and during summer (126%).

The relationship between moisture content and instrumental firmness was evaluated through the Pearson correlation and the results were presented in Figure 4 (A-F). All analyzed data showed a negative correlation, the higher the moisture content, the lower the firmness, with high correlation coefficients (r) and also high coefficients of determination (r^2), except the data from P1 during winter, although only the data from P3 during winter were significant, with 95% confidence ($p < 0.05$).

4. Discussion

Artisanal cheeses are considered unique as their sensory diversity derives from the lack of standardization of the types and concentration of microorganisms involved in the manufacture of the product (Bachmann et al., 2011), as well as the different environmental characteristics which influence the proteolysis, among other transformations. The proteolysis can be catalyzed by enzymes from different origins: (i) produced by the microbiota naturally present in the raw milk, (ii) from added microorganisms, (iii) naturally present in raw milk and/or (iv) from the milk coagulation agent (McSweeney, 2004; Bachmann et al., 2011).

4.1. TRICINE-SDS-PAGE

Casein is the main constituent of cheese and can be classified according to composition and amino acid sequence into four fractions: α_{S1}, α_{S2}, β and κ-casein (Perna et al., 2014). The manufacture of all varieties of cheese involves the coagulation of the casein. For enzymatic coagulation, a network that retains the milk fat occurs. During coagulation, κ-casein is hydrolyzed in the primary phase of rennet action and loses its protective capacity, resulting in the release of the hydrophilic C-terminal segment and in the formation of small peptides during coagulation. When pure renin is used as the coagulant, peptide bond hydrolysis occurs between amino acids Phe-105 and Met-106 (Fox et al., 2004). The proteolytic activity during cheese ripening depends on several factors, such as the type of coagulant used, residual action of the coagulant and native milk proteases, which may be influenced by the moisture content of the cheese as well as the temperature and relative humidity of the ripening place (Fox et al., 2015)

Different casein fractions and protein breakdown products can be observed throughout ripening by electrophoresis, which is probably one of the most used techniques to monitor the cheese ripening process (Perna et al., 2014). The α-casein fractions are more susceptible to proteolysis whereas the degradation of β-casein is much less common (Fox et al., 2004). Electrophoresis techniques are based on the separation of proteins according to molecular weight and allow the comparison of the intensity of the stained polypeptide chains in a polyacrylamide gel.

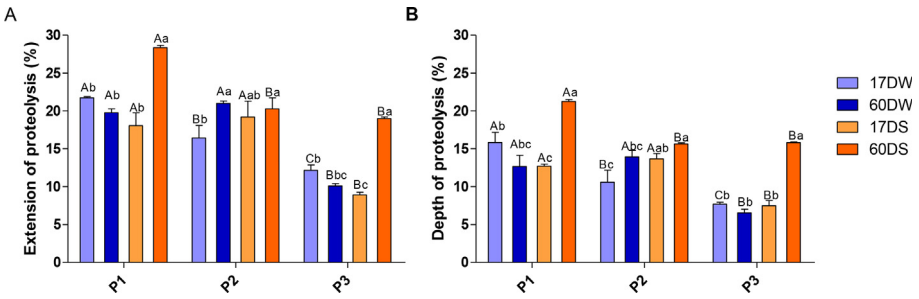

Figure 2. Ripening Extension (A) and Depth (B) indices (%) during winter (W) and summer (S). Different uppercase letters indicate a significant difference between the different producers (P1, P2, P3) at the same time of analysis. Different lower-case letters indicate significant difference over time and seasons for the same producer.

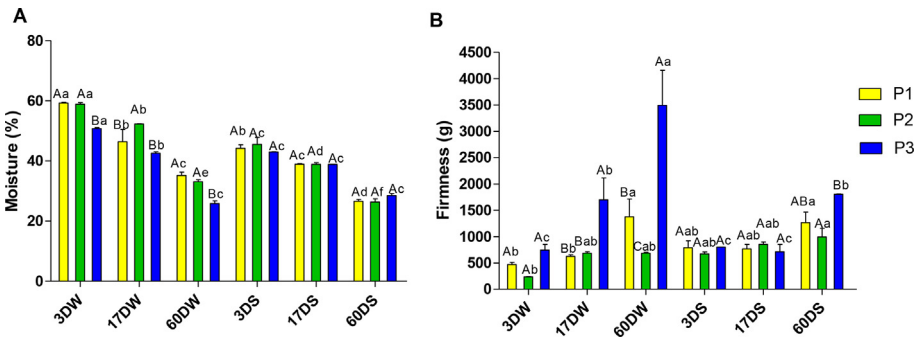

Figure 3. Moisture (A) and instrumental firmness (B) during Winter (W) and Summer (S). Different uppercase letters indicate a significant difference between the different producers (P1, P2, P3) at the same time of analysis. Different lower-case letters indicate significant difference over time and seasons for the same producer.

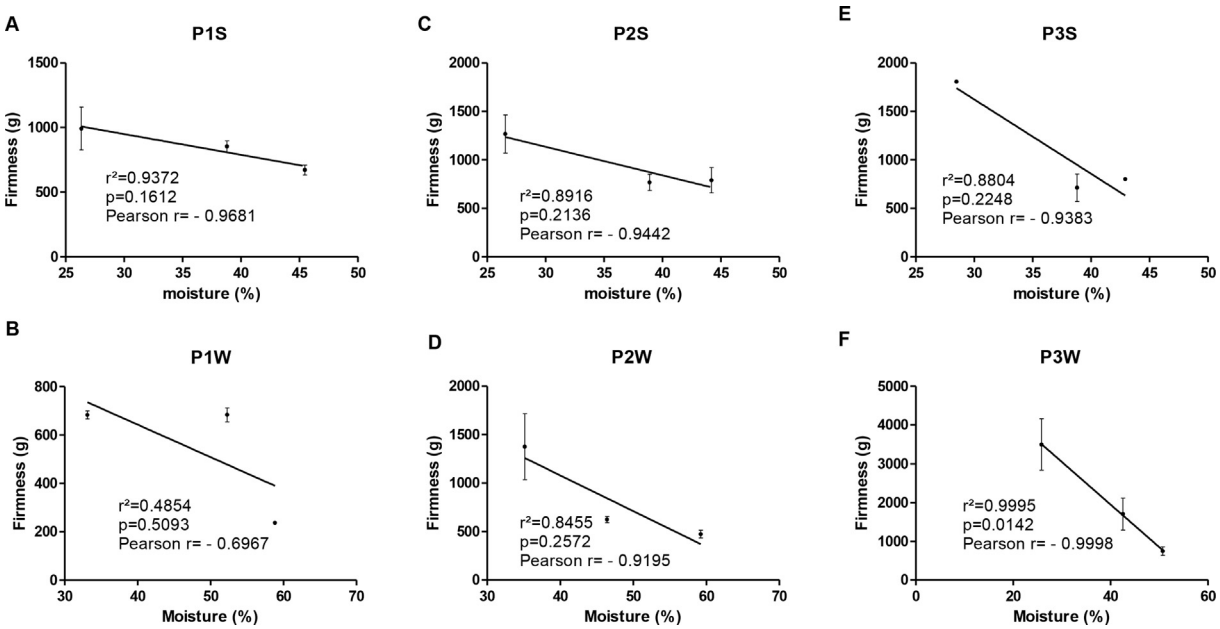

Figure 4. Pearson's correlation for moisture (%) and firmness (g). Where P1; P2; P3 are the three producers; S means summer; W means winter. Graphs A and B show the results for producer P1 in summer and winter, respectively. Graphs C and D present the results for producer P2 in summer and winter, respectively. Graphs E and F show the results for producer P3 in summer and winter, respectively.

Electrophoresis can be used to monitor casein hydrolysis into smaller compounds, to evaluate the formation of peptides of different molecular weights, which helps the understanding of the proteolysis that occurs during cheese ripening (Zhao et al., 2019).

The TRICINE-SDS-PAGE results in this study showed that proteolysis could be related to the duration of ripening, as no sample showed any signs of proteolysis after three days of maturation, and as it was possible to verify higher intensity of low weight bands (peptides) over the ripening time (Figure 1).

Proteolysis could also be related to the season of production, as higher hydrolysis was detected in summer than in winter. During summer, temperatures and relative humidity are higher, which may influence the activity of proteases. But mostly electrophoresis showed the relationship between proteolysis and the producer of origin of the samples. This was expected, to some extent, as the endogenous microbiota present in the *pingo* and in the raw milk was characteristic of each property and it results in the typical *Terroir* of each producer. However, the behavior of the samples from P3 suggested that other factors may also have exerted some influence. In the manufacturing of AMC-Serro, salting is performed on the surface of the cheese and whey drainage occurs for three days, while the cheeses are in the molds. On the third day, the product is unmolded and placed on wooden shelves in the ripening room to begin maturation. During the ripening, the cheeses may be rinsed with running water, to remove surface fungi that may develop, and the thickening of rind occurs. Washing occurs every two or three days, depending on the ambient temperature and humidity conditions of the ripening room (Chaves et al., 2018). The producer #3 was the only one in this study to wash periodically the rind of the ripening cheeses, thus preventing the growth of fungi on the surface. Unlike P3, the producers #1 and #2 did not wash the cheeses during the ripening, allowing the development of mold on their surfaces. It is possible that the metabolites of these fungi have penetrated the interior of the cheeses and influenced the proteolysis, which would explain the differences observed in the electrophoretic profile of the cheeses studied. It was also observed that the P1 samples showed similar behavior in the two seasons studied, probably due to the characteristics of temperature in the maturation room of this producer, that showed the least variation throughout the year among all producers.

The differences in the electrophoretic profile of the 3 producers attest that there is a lack of standardization among their processing units. Future supervision should be given to these producers in order to guide them to take measures to minimize the differences.

4.2. Ripening extension and depth indices

The evaluation of the ripening process in cheeses may be achieved by the analysis of two ripening indices: the ripening extension index and the ripening depth index, where the soluble nitrogenous substances that accumulate, due to casein hydrolysis, are quantified. The ripening extension index reflects the amount of proteins and peptides that are soluble in water at pH value of 4.6 - the isoelectric point of caseins, which, when intact, remain insoluble. The ripening depth index indicates the amount of low molecular weight nitrogenous substances (free amino acids, small peptides, among others) accumulated during ripening, that remains soluble in a 12% TCA solution (Pereira et al., 2010).

The ripening extension index is related to primary proteolysis, associated with the action of milk endogenous proteases and the enzymes used as coagulants on α_S-caseins and, to a lesser extent, on β-casein, giving rise to high and medium molecular weight peptides (Roseiro et al., 2003; Fox et al., 2004). Since all producers used the same commercial coagulant from the same supplier, the differences found between the samples for the proteolysis extension index, in the same season, should be credited to the differences due to the endogenous milk proteases or proteases produced by the microorganisms present of each producing farm. Milk has some endogenous proteases, among which plasmin is the main one. It is an alkaline protease, which preferably hydrolyzes β-casein to γ-casein (Fox et al., 2004; Guerreiro et al., 2013).

Also, as very little proteolysis was detected during winter, it is reasonable to infer that the activity of these proteases was favored by the higher temperature, characteristic of the summer season, as has already been stated by other authors for Mozzarella cheese (Guinee et al., 2001; Costa Junior et al., 2009). This may also explain why the samples from P2 showed less extension of proteolysis, even in the summer: as the ripening room in this farm is thoroughly protected from the sun, the temperature during the ripening period remained lower over the whole year. It is also noteworthy that the practice of washing the cheese rind throughout maturation, characteristic of this region and applied only by producer #3, seems to have led to a lower ripening extension index, especially in winter, when compared to producers #1 and #2. It is possible that this practice contributed to causing a reduction in the cheese temperature, reducing the enzymatic activity.

The ripening depth index represents mostly the presence of smaller peptides (from 2 to 20 amino acids) which are formed by the further hydrolysis of the larger peptides generated by the coagulant proteases in the first stages of maturation. In general, the enzymes secreted by the microbiota are responsible for this stage of proteolysis (Fox et al., 2015; Pereira et al., 2008). The results for this index showed the same behavior and profile as the results obtained for the extension index, probably for the same reasons. However, for P3, the washing process may also have interfered in the growth of superficial microorganisms, hindering even further the proteolysis during winter. It is remarkable, nonetheless, that this procedure did not prevent proteolysis in the cheeses from P3 to reach the same levels as the samples from P2, in the summer, probably due to the higher temperature that was achieved in the ripening room, exposed to the afternoon sun, between washes.

AMC-Serro studied by Pinto et al. (2017) presented proteolysis extension values around 12% and close to 7% for proteolysis depth, after 8 days of ripening, which are within the range observed in this study for the samples from P3, also a typical rind washed AMC-Serro producer.

Several regions in the state of Minas Gerais produce AMC like the ones produced in Serro. Cheeses produced in these regions were evaluated in studies by Costa Junior et al. (2009) and Silva et al. (2011), who studied the AMC-Canastra, and Sobral et al. (2015) who studied the AMC-Araxá and AMC-Cerrado, respectively. In these studies, cheeses presented ripening extension indices of 13.55% (Araxá), 13.14% (Cerrado) and 10.64% (Canastra). The ripening depth indices were 8% (Araxá), 8.94% (Cerrado) and 4.66% (Canastra). The AMC-Canastra were ripened for 8 days and AMC-Araxá and AMC-Cerrado were evaluated for up to 60 days of ripening. It was observed that proteolysis increased over the ripening time for the AMC of the different regions studied.

4.3. Moisture and firmness

According to Resolution No. 7 (Brasil, 2001), which regulates and supervises the manufacture of artisanal cheeses in Brazil, AMC are supposed to be medium-moisture cheeses, with moisture content between 36.0 and 45.9%. In this study, all cheese samples produced in summer presented moisture values within this range, from the beginning of the ripening process. However, after 60 days of ripening, regardless of the season, all samples were classified as low-moisture cheeses, with moisture content under 36%. More concerning was the finding about the water loss speed during the winter. According to the same Resolution (No. 7), AMC must be matured for at least 17 days, period during which the cheeses must reach the regulatory moisture, that has the purpose to ensure the safety and the quality of the product. The samples from P1 and P2 showed moisture contents higher than 45.9%, after 17 days of ripening, in winter, qualifying as high-moisture cheeses. Water loss for the samples from P1 and P2 did not change with the seasons, probably due to the location of the maturation rooms on these farms, sheltered against the sun and, thus, with less variation in temperature and relative humidity throughout the year. As the samples showed higher initial moisture in winter, it was not possible to reach the desired final moisture after only 17 days of ripening, during this season. Machado et al. (2004), when analyzing AMC-Serro after 6 days of ripening during winter, also classified them as high-moisture cheeses. The samples from P3 showed a somewhat unexpected behavior, sustaining higher water loss in winter than in summer, which may be related to the proteolysis that occurred differently during the two seasons: proteolysis was very limited in the samples from P3 in winter but not in summer.

There is a correlation between cheese firmness and the amount of intact α_S-caseins present, which is explained by the fact that casein decomposition products are mostly water soluble and therefore do not contribute to the protein matrix. In addition, each peptide bond that is cleaved generates two new ionic groups that compete for the available water. Thus, water previously used for protein chain solvation is bound to the new ionic groups (Lamichhane et al., 2018). As a consequence, high levels of proteolysis tend to decrease water loss during ripening as well as to reduce cheese firmness over time.

Instrumental firmness determines shear strength, measured at maximum force and expressed in grams (Marinho et al., 2015). Cheese texture is largely dependent on the relationship between casein and moisture (McSweeney et al., 2006; Zhao et al., 2019). In the present experiment a fairly good negative correlation between water loss and firmness could be verified: as a decrease in moisture was observed, the cheese samples became increasingly resistant to deformation, that is, firmer. The same can be said about proteolysis. If the extent of proteolysis (Figure 2A) is compared with firmness (Figure 3B), for all producers, hydrolysis was higher in summer and firmness was lower in the same season, in a negative relationship, as expected. Likewise, there was a lesser water loss when there was greater hydrolysis, for all producers.

5. Conclusion

The present paper shows the first attempt, to the extent of our knowledge, to relate the proteolysis during ripening of AMC-Serro with the different producing farms and seasons. In general, proteolysis was more intense in summer than in winter, due to the higher temperatures and moisture, which influence the enzymatic activity in the cheese. Other factors directly or indirectly influencing the temperature also determined the behavior of proteolysis, such as ripening room location and rind-washing, showing large differences among the three producers evaluated. The degree of proteolysis also influenced the water loss during ripening, with great influence on cheese safety.

The results obtained in this study may be used to better understand the transformations during ripening of AMC-Serro and help the small traditional farmers improve their product's quality and stability.

Declarations

Author contribution statement

Juliana de Oliveira Carneiro: Performed the experiments; Wrote the paper.

Ana Carolina Sampaio Doria Chaves, Cleube Andrade Boari: Conceived and designed the experiments.

Marilia Penteado Stephan: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Maria Gabriela Bello Koblitz: Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported by Embrapa (001).

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e04446.

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