Journal of Food Technology 12 (3): 66-72, 2014
ISSN: 1684-8462

A Study of the Physico-Chemical, Electrophoretic and Rheological Properties Throughout Ripening of Sao Jorge (PDO) Raw Cow's Milk Cheese

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Abstract: Texture is an important quality parameter that determines the identity of a cheese and greatly affects its consumer preference and acceptance. In the present study, the texture (rheological properties), physicochemical and proteolysis profiles of Sao Jorge cheese at 90, 120 and 210 days of ripening were evaluated. The moisture content ranged between 36-37%, the pH values lay between 5.20 and 5.45 with highest values found consistently in 120 days samples ($p>0.05$)and the instrumental hardness varied between 550 and 700 g. As cheeses aged, the total protein varied from 25.89 g/100 g in 90 days cheeses to 27.41/100 g in 210 days samples; the protein electrophoretic patterns showed a consistence between cheese age and a lowering in intensity of bands associated to intact casein and conversely an increase in intensity and number of bands associated to the water-soluble extracts components, commonly used as index of cheese ripening. It was concluded that Sao Jorge cheeses of 120 days are markedly different from their counterparts of 90 and 210 days, being characterized by a slight higher moisture, higher pH value, higher stress-strain slope, lower hardness and a lower resistance to compression, indicative of a more fragile structure. This is the first systematic report on the evolution of texture in Sao Jorge cheese.

Key words: Sao Jorge cheese, rheology, hardness, compression, electrophoresis, proteolysis

INTRODUCTION

Sao Jorge cheese is a Portuguese traditional raw-milk cheese that bears the Protected Designation of Origin (PDO) distinction. It is the most important source of income for the islanders in Azores archipelago where it has been made for >500 years (Kongo, 2010). Sao Jorge cheese is produced from grass fed cow's raw-milk via coagulation at 30-31°C, using a natural whey-starter culture, typically ripening for 3-7 months, after which the cheese weigh ca 8-15 kg, exhibit a cylindrical shape, irregular eyes and a unique spicy flavor (Kongo *et al*., 2009; Kongo, 2010). In the aging room where they are required to stay for a minimum of 90 days, the cheeses are arranged on wooden shelves and turned frequently. At the end of said period the cheeses are tasted by members of the Confraria do Queijode Sao Jorge who indicate whether the cheeses have aged as expected and thus are able to be branded with the PDO seal of Sao Jorge or recommend it to be sold without said seal. Today Sao Jorge cheese has found a wider market such as in USA and Canada and consumers may enjoy the younger cheeses (3 months) or older cheeses which exhibit a typical stronger and piquant taste.

Although, the chemical and microbiological changes throughout ripening of Sao Jorge cheese have been previously studied (Kongo, 2010), the texture profiles, are still little known. Recall that texture is an important quality parameter that determines the identity of a cheese and greatly affects its consumer preference and acceptance (McEwan *et al*., 1989; Guinard and Mazzucchelli, 1996). Because consumers' preference is low whenever the perceived texture during mastication does not match the expectations, it is crucial to understand how the texture of a cheese changes during ripening. This makes improvements possible, leading consistently to cheeses with typical taste and texture and thus maintaining consumers' loyalty.

Cheese texture is defined as all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical, tactile and where appropriate, visual and auditory receptors

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(Muthukumarappan and Karunanithy, 2011). Texture is affected by the processing techniques and storage conditions (Konstance and Holsinger, 1992) due to the effect they have on cheese composition, (pH, amount of fat and the ratio of intact casein to moisture, (Lawrence *et al*., 1987) and on the rate of proteolysis-an important event in the ripening of most cheese varieties that simultaneously influences the textural and flavour characteristics of a cheese (Grappin *et al*., 1985; Rank *et al*., 1985; Visser, 1991; Wium, *et al*., 1998). In general, cheese texture will change markedly in the first 1-2 weeks of ripening as the hydrolysis of a small fraction of α_{s1}-casein by the rennet to the peptide α_{s1}-I results in a general weakening of the casein network. The following and usually relatively slow change in texture is often associated to rate of proteolysis which in turn is controlled largely by the proportion of residual rennet, plasmin, microflora peptidases, salt to moisture ratio and storage temperature (Lawrence *et al*., 1987). Thus, the early stage hydrolysis (primary proteolysis) that leads to formation of large specific peptidesand the later stage proteolysis (secondary proteolysis) occurrs when said peptides are digestedinto smaller ones and even free AA by enzymes from starter or nonstarter microorganisms (Benfeldt and Sorensen, 2001). Both play an important role for the development of the final texture in ripened cheeses (Fox *et al*., 2000). Several criteria, including nitrogen solubility in a range of solvents rheological measurements and total protein measurements via Kjeldhal Method may be used to determine the indices of proteolysis in cheese but electrophoresis is, arguably, the most useful and widely used method (Shalabi and Fox, 1987).

One of the most important texture attributes is hardness, often used to compare food samples (Dan *et al*., 2008) and defined as the force required to penetrate (bite on) the sample with the molar teeth (Lee *et al*., 1978; Bryant *et al*., 1995; Hort and Le Grys, 2000; Gunasekaran and Ak, 2003; Dan *et al*., 2008). As such a property is not easily quantifiable using human subjects, an instrumental hardness is determined, normally using a cylindrical probe to estimate the force needed to penetrate the cheese samples. Hardness has been quantified in several cheeses such as Parmesan, Edam, Gouda, Swiss, Cheddar, Mozzarella, Provolone, Muenster, Colby and Brick cheese (Chen *et al*., 1979); goat cheese (Sanchez-Macias *et al*., 2010); Petit-Suisse cheese (Souza *et al*., 2011) and processed cheese spreads (Kycia *et al*., 2006). Cheese rheology studies are often undertaken via application of uniaxial compression using a universal testing machine (Antoniou *et al*., 2000; Goh *et al*., 2003; Wium *et al*., 2003; Kycia *et al*., 2006; Madadlou *et al*., 2007, 2006; Maruyama *et al*., 2006; Rogers *et al*., 2009). The texture and taste of a cheeses will obviously change with aging and said parameters may show an undesirable variability in artisanal cheeses due to a more empirical and less sophisticated processing technology. Thus, knowing the right characteristics of the cheeses at a specific period of ripening may help in devising processing improvements towards obtaining the right product consistently as well as better meeting consumers' desire and eventually their fidelity.

The main objective of this study was to pursue a first systematic approach in studying the evolution of texture of Sao Jorge cheese via analysis of the rheological properties (hardness and uniaxial compression), the electrophoretic and total protein analysis during the different ripening times (90, 120 and 210 days).

MATERIALS AND METHODS

Samples: Sao Jorge cheese samples (n = 21-seven for each of the ripening time, i.e., 90, 120 and 210 days) were purchased from the local market. A representative portion of each cheese was obtained by cutting a cheese cylinder via inserting a cheese trier into the cheese wheel. Then, the outermost part (crust) was removed from the ends and the remaining sample was used according to each analysis, after the samples were left at room temperature for 3-4 h prior to testing.

Physicochemical analysis: Cheese samples were grated and prepared prior to analysis for moisture according to ISO 5534:2004. All samples were analyzed in duplicate. A Hanna pH shear (series 1412679, England) was used for measuring the pH of cheese samples by direct insertion of a solid pH electrode that had been previously calibrated. Total fat content (%) was measured according to Portuguese Norm 2105:1983 using the Gerber Method.

Rheological analysis

Textural evaluation (hardness): The hardness of the samples was performed using the methods of Chen *et al*. (1979) and Sanchez-Macias *et al*. (2010) with some modifications. Texture was analyzed using a HTE Universal Testing Machine (Instron model 4501, series H3279, England) with a 5000 N load cell. A cylindrical probe with 5 mm diameter was attached to the crosshead. The samples were cut into rectangular shapes using a sharp razor (20 mm high, width 55-60 mm and length 45-60 mm), immediately placed in airtight containers to prevent dehydration and analyzed at room temperature. Prior to texture analysis, a 5 mm layer was removed from the upper surface of each cheese to obtain a regular surface. The speed of the crosshead was set at 40 mm min^{-1}. The cheese sample was placed on a flat holding plate. The penetration of the cylindrical probe into the cheese sample was set for 35 mm (high extension) from the initial height. Eleven penetrations were performed in each piece in a total of fourteen pieces (n = 154).

Uniaxial compression: For uniaxial compression Sao Jorge cheese samples were prepared following the method described by Tunick (2000) with some modifications. The test was performed using a HTE Universal Testing Machine (Instron Model 4501, series H3279, England) with a 5000 N load cell. A flat plunger with a 35 mm diameter was attached to the moving crosshead. Cheese blocks were cut into cylinders (30 mm diameter and 10 mm high), immediately placed in airtight containers to prevent dehydration and analyzed at room temperature. Samples were compressed uniaxially at a crosshead speed of 50 mm min^{-1} to 70% deformation (test end-point: 7 mm) from the initial height of the sample in one bite. To analyze the compression curves resulting from the uniaxial compression of samples, engineering stress ($\sigma\mu$), Hencky strain (εh) and the modulus of deformability were calculated according to ISO (2006).

Electrophoretic analysis of cheeses: The Water-Insoluble Extracts (WISE) and Water-Soluble Extracts (WSE) were obtained by the procedure of Kuchroo and Fox (1982); the cheese-like systems were homogenized in a stomacher at 20°C for 10 min with twice its weight of water then the slurries were held at 40°C for 1 h, centrifuged and filtrated. The retentate (WISE) and the filtrate (WSE) were stored at -80°C and finally freeze dried. Before analysis 2 mg of each sample was dissolved in 800 μL of a Tris–HCl, pH 6.8 buffer in the presence of 0.1% of SDS and 5% of mercaptoetanol, heated to 100°C por 3 min and 200 μL of a solution of 10% glycerol. Finally 0.01% de bromophenol blue was added before application to a gel (Egito *et al.* 2006). The electrophoresis was performed by running the gels at 90 V for 2 h, in a mini-protean III dual slab cell (Bio Rad Laboratories, Watarford, UK). Immediately after ending the electrophoresis, gels were placed in a fixative solution containing 50% methanol and 10% acetic acid for 2 h and then stained with a solution containing 10% acetic acid and 0.1% CoomassieBrillant Blue in which the gels were left for 30 min at room temperature. The gels were then unstained with at least 3 changes of distillated water in a period of 24 h.

Determination of total protein: The modified AOAC Method 920.123 for the determination of Total Nitrogen (TN %) in cheese by Kjeldahl analysis was used (Lynch *et al.*, 2002) by analyzing each sample in triplicate. Subsequently, the total (crude) protein of the samples was calculated as %TN×6.38.

Statistical analysis: Results are expressed as mean± standard deviation. The Analysis of Variance (ANOVA) was carried out using the SPSS. Tukey's test was used to evaluate the differences between means (level of significance was determined at $p<0.05$). All the charts and basic calculations were made using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Physicochemical analysis: The average values of pH, moisture and fat of samples of 90, 120 and 210 days of ripening are shown in Table 1. While the three factors affect cheese texture (Gunasekaran and Ak, 2003), the role of pH is particularly crucial because changes in pH are directly related to chemical changes in the protein network of the cheese curd which will also affect proteolysis in later ripening phases (Lawrence *et al.*, 1987). The pH values of Sao Jorge cheese samples lay between 5.20 and 5.45 with highest values found consistently in 120 days samples, decreasing slightly in 210 days cheeses samples ($p>0.05$). This pattern, also observed else where, may explained by the fact that in Sao Jorge cheese there is a more intense proteolysis from 90-120 days resulting in build-up of free amino acids and even ammonia which tend to increase the pH of the cheesesby 120 days (Kongo *et al.*, 2009). Malacarne *et al.* (2006) also found that in Parmigiano-Reggiano cheeses the intensity of proteolysis diminishes in later phases of ripening (Table 2). The reduction of insoluble Calcium (Ca) is also reported to cause an increase of the pH (Kosikowski and Mistry, 1997; Kongo *et al.*, 2009) as when Ca is solubilized, phosphate groups are released from colloidal

Table 1: Composition (average±standard deviation, n = 4) of Sao Jorge cheese by 90, 120 and 210 days of ripening

Ripening time (days)	Parameters Moisture (%)	Fat content (%)	pH
90	36.19±0.93[a]	34.5±0.4[a]	5.27±0.09[a]
120	36.78±0.66[a]	32.5±0.3[b]	5.43±0.04[b]
210	36.63±1.29[a]	32.0±0.6[b]	5.36±0.03[b]

Different letters represent means with statistical differences for $p<0.05$

Table 2: Total average protein content of Sao Jorge cheese samples throughout the ripening time (90, 120 and 210 days)

Ripening time (days)	Total protein content (g/100 g)
90	25.89±0.12[a]
120	26.65±0.58[ab]
210	27.41±0.05[b]

Different superscript letters represent mean values that are statistically different for $p<0.05$

Ca phosphate and this phosphate combines with hydrogen ions decreasing the concentration of free hydrogen ions in cheese which results in an increase in cheese pH (Hassan *et al*., 2004). Roefs *et al*. (1985) pointed out that with pH values between 5.1 and 5.5 there is an increase in colloidal phosphate and considerable casein dissociation from the submicelles. These changes in the size and characteristics of the submicelles significantly increase their ability to absorb water (Lawrence *et al*., 1987) affecting cheese texture. In general, these results agree with those found by Kongo *et al*. (2009) and the pH values obtained were similar to those for Muenster, Provolone, Brick and Parmesan cheeses (Chen *et al*., 1979).

The average values of moisture content in the cheeses samples is shown in Table 1 and they ranged from 36-37% while Kongo *et al*. (2009) reported values ranging in 38-42% in Sao Jorge. In present research, the Tukey test of means suggests that moisture is essentially similar from 90-210 days of the ripening process ($p>0.05$). The fat content in cheeses of 120 and 210 days were found to be significantly different from those of 90 days samples (Table 1) which essentially agrees with previous results (Kongo *et al*., 2009).

Rheological analysis

Textural evaluation (hardness): Texture, particularly in cheese is one of the most important attributes to determine the identity of a product (Tunick, 2000). Hardness of cheese is defined when a specimen is compressed between the molar teeth (Szczesniak, 1987). To measure cheese hardness, only the force required to break the cheese surface would have to be known (Weik *et al*., 1958). Figure 1 shows the average±standard deviation of hardness obtained for Sao Jorge cheese during its ripening time. Sao Jorge cheese samples exhibited hardness values ranging from 500-750 g which decreased significantly ($p<0.05$) from 90-120 days of ripening time, increasing afterwards from 120-210 days. Tukey's test indicates that statistically, the 90 and 210 days cheeses belong to the same hardness group while curiously, 120 days Sao Jorge cheese belong to a separate group. The lower hardness found in 120 days may be explained by the combination of two factors, namely, a high rate breakdown of a_{s1}-casein and the consequent rise in pH from 90-120 days which together with fat and moisture contents significantly affect cheese hardness (Weik *et al*., 1958). The higher moisture contents in 120 days although statistically not significant

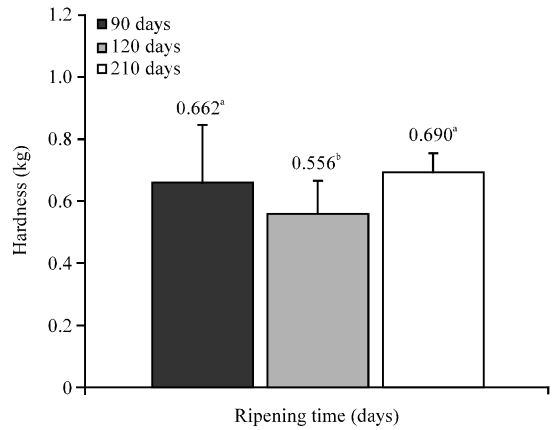

Fig. 1: Hardness of Sao Jorge cheese (average±standard deviation, n =154) by 90, 120 and 210 days of ripening. Different superscript letters represent mean values that are statistically different for $p<0.05$

different ($p>0.05$), combined with the corresponding higher pH values may partially elucidate the observed decrease in hardness in said samples.

O'Mahony *et al*. (2005) proposed that the initial softening of texture could largely occur due to the solubilization of insoluble Ca as seen in Cheddar cheese. Contrary to finding by Chen *et al*. (1979), in the present research the increase in the pH values did not result in higher cheese hardness but rather in a decrease in hardness, as show in Fig. 1. Among the eleven cheeses analyzed by Chen *et al*. (1979), in terms of hardness, Brick cheese was the most similar to Sao Jorge (ranging between 500 and 700 g) while on the other hand, cheeses like Parmesan, Gouda or Cheddar are totally different from Sao Jorge since the instrumental hardness is much higher than the observed in the present research.

Uniaxial compression: The uniaxial compression characterizes a cheeses deformation behavior when subjected to stress or strain (Guinee, 2002). Typical stress-strain curves of Sao Jorge cheese compression at 70% are shown in Fig. 2. The average slope (Modulus of Elasticity-E) obtained from the stress-strain curves are shown in Fig. 3. Sao Jorge cheese of 120 days of ripening exhibited a modulus of deformability higher than the other samples. Again, 90 and 210 days cheeses show a more similar modulus of elasticity compared to that of 120 days cheese samples. It is notorious that Sao Jorge cheesesof 120 days are different as they are characterized by a slight higher moisture, higher pH value, higher stress-strain slope, lower hardness and a lower resistance

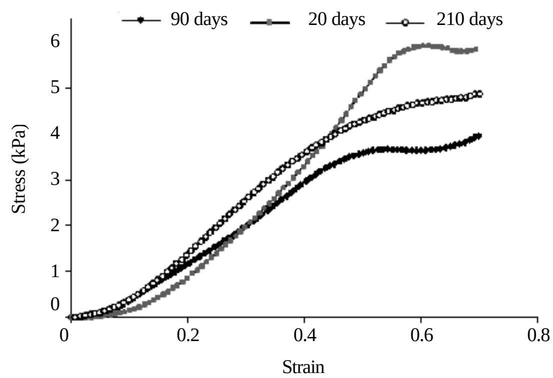

Fig. 2: Stress-strain curves of Sao Jorge cheese with 90, 120 and 210 days of ripening

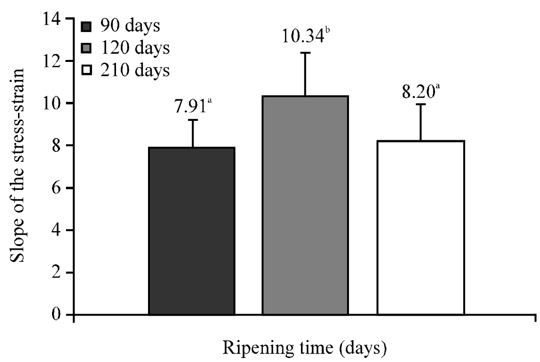

Fig. 3: Modulus of elasticity obtained from the stress-strain curves of Sao Jorge cheese (average±standard deviation, n = 154) by 90, 120 and 210 days of ripening. Different superscript letters represent mean values that are statistically different for $p<0.05$

to compression, indicative of a more fragile structure. Furthermore, Pearson correlation coefficient between hardness and modulus of deformability (R^2 = -0.953; p = 0.098) shows that higher hardness means lower stress-strain slope and consequently, higher resistance to compression for 90 and 210 days cheeses.

The analysis of the compression at 70% deformation of the initial Sao Jorge cheese height was achieved after finding the apparent fracture point of each sample. Fracture point is defined as the local maximum of the compression curves just before the samples squash (ISO, 2006). According to Antoniou *et al.* (2000) compression at 10% deformation evaluate better the cheese texture than the 80% compression mainly because it ensures that only few secondary bonds are destroyed when cheese samples are compressed and the structure can be easily recovered. In the 80% deformation most of the primary bonding is ruptured and the fracturing of

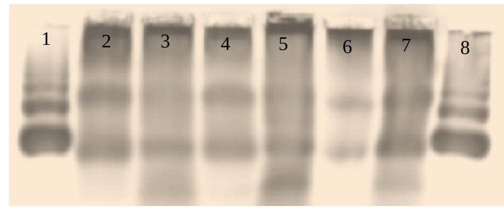

Fig. 4: Electrophoretograms of Sao Jorge cheeses samples at 90, 120 or 210 day ripening: Lines 1, 8-Bovine Albuminestandard (BSA); Lines 2, 3-Water-Insoluble Extract (WISE) and Water-Soluble Extract (WSE), respectively, of 90 days cheeses; Lines 4, 5-WISE and WSE, respectively, of 120 days cheese samples; Lines 6, 7 WISE and WSE, respectively of 210 days cheese samples

the structure is not only extensive but also uncontrolled. Also, minor differences that distinguish the texture of one variety from the other are easily ignored. Therefore, it can be said that the 10% compression measurements of the cheese samples are more sensitive than the 80% compression applied.

Electrophoretic analysis: Although, several criteria may be used as indices of proteolysis in cheese electrophoresis is, the most useful and widely used method (Silva and Malcata, 2005). Figure 4 shows the electrophoretograms of Sao Jorge cheeses analyzed in the present research. It can be observed that band intensities of WISE (associated to intact casein or large peptides derived there from) decrease with the ripening time while those of WSE (associated to small peptides and amino-acids formation) in general increase with ripening. These observations are consistent with the occurrence of preoteolysis and degradation of casein to peptides of smaller size throughout ripening of Sao Jorge cheeses. These results agree with previous observations reporting a high proteolytic activity during Sao Jorge cheese ripening leading to formation of a high concentration of peptides eluting in the hydro-phobic region by 130 days (Kongo *et al.*, 2009). As expected, the densitometric analysis of all gels also showed a higher number of peeks for WSE gels (as compared to WISE gels) indicating the presence of nitrogen compounds most of which are commonly used as index of cheese ripening (Grappin *et al.*, 1985; Rank *et al.*, 1985; Fox *et al.*, 2000).

Crude protein analysis: While the values obtained for total protein were in the range of those indicated by Lynch *et al.* (2002), 120 and 210 days of ripening Sao Jorge cheeses had crude protein values higher than the average values indicated by those researchers.

CONCLUSION

The texture of Sao Jorge cheese samples at different ripening periods was accurately determined using instrumental techniques. The compression and texture measurements were useful to understand the structure and characterize differences between ripening times (90, 120 and 210 days). The results showed that cheeses of 120 days are markedly different as they are characterized by a slight higher moisture, higher pH value, higher stress-strain slope, lower hardness and a lower resistance to compression, indicate a more fragile structure than its counterparts of 90 and 210 days. Said cheeses may be preferred by a different niche of consumers and specific marketing strategies may thus be undertaken aiming those consumers to potentially increase their fidelity and ease of choice.

The patterns exhibited by the electrophoretograms indicate a consistent preoteolytic activity as Sao Jorge cheeses ripens which corresponds with the intense taste and aroma that consumers in general attribute to aged Sao Jorge cheeses.

ACKNOWLEDGEMENTS

This research is part of the SEPROQUAL INOVACAO project supported by the program Proconvergencia, the Government of Azores and Secretaria Regional para a Ciencia. Researchers would like to thank Dr. Pedro Louro (INRB-Institut oNacional de Recursos Biologicos, I.P.) for his insights on cheese rheology.

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