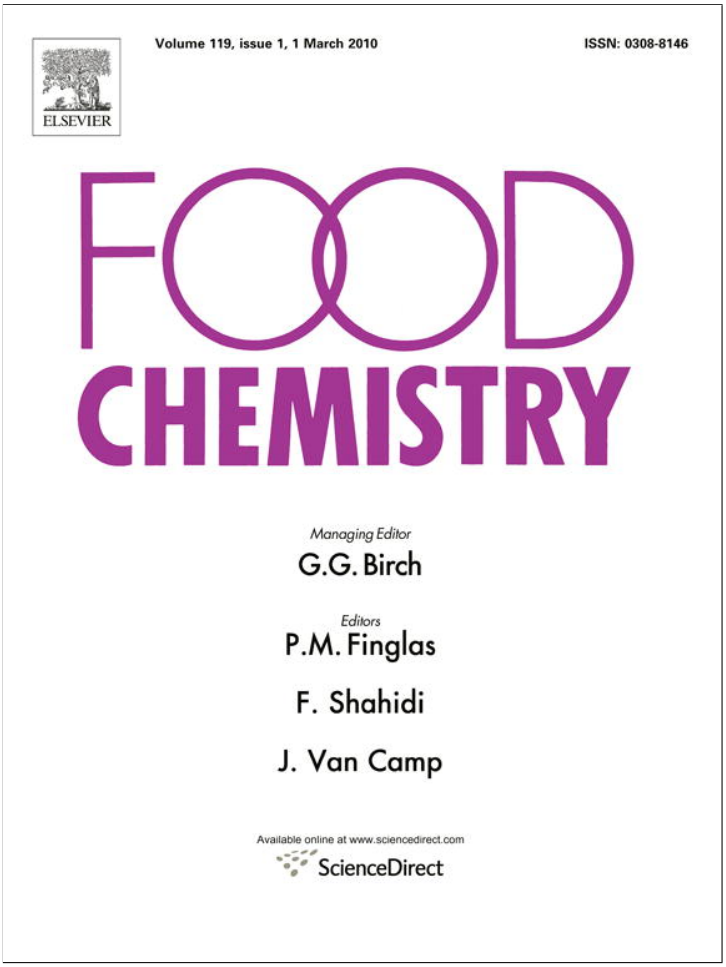

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Acceleration of cheese ripening at elevated temperature. An estimation of the optimal ripening time of a traditional Argentinean hard cheese

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ABSTRACT

The effect of elevated ripening temperature on physicochemical, biochemical and sensory characteristics in Reggianito Argentino cheese was evaluated to determine the optimal time for cheese ripening at 18 °C that ensures typical cheese characteristics. Cheeses ripened at 12 or 18 °C and 85% relative humidity were analysed at 2, 4 and 6 months. Seventy-eight variables (as determined by urea-PAGE, RP-HPLC of the water-soluble at pH 4.6 fraction, free amino acids, free fatty acids and sensory analysis) were considered for the principal component analysis. The statistical analysis allowed determination of the optimal time for ripening Reggianito Argentino cheese at 18 °C, which was ranged between 2 and 3 months. In conclusion, the results obtained were not only useful in characterising the ripening of an Argentinean hard cheese, but also in evaluating the effect of an increase of ripening temperature on the main physicochemical, biochemical and sensory changes of Reggianito Argentino cheese.

1. Introduction

Ripening is a slow and consequently an expensive process that is not fully predictable or controllable. Consequently, there are economic and possibly technological incentives to accelerate ripening (Fox et al., 1996). Azarnia, Robert, and Lee (2006) analysed different alternatives to accelerate the maturation of Cheddar cheese and their economic and technological implications. The authors concluded that methods such as addition of enzymes, adjuncts, attenuated cultures or genetically modified starters are associated with particular drawbacks (poor availability of commercial enzyme preparations, difficulty in uniformly distributing them in the cheese matrix, etc.), which make their implementation difficult. Other methods such as the use of enzyme encapsulation or liposomes are inefficient or expensive. The authors reported that although the elevated ripening temperature poses a risk in terms of the growth of unwanted microbial contaminants, it may be safely used when cheese is made with pasteurised milk under good manufacturing conditions.

Undoubtedly, elevated ripening temperatures offer the most effective, and certainly the simplest and cheapest, method for accelerating ripening. However, considering the numerous complex biochemical reactions that occur during ripening, it is unlikely that all processes will be accelerated equally at elevated temperatures and unbalanced flavour or off-flavours may result (Fox et al., 1996). Therefore, when an elevated ripening temperature is used, it is necessary to evaluate the different areas related to cheese ripening.

Reggianito Argentino cheese is the most important hard cheese variety manufactured in Argentina, and it is exported to different countries. Italian immigrants in the late 19th and early 20th centuries inspired by Italian hard cheeses, developed it, and later the technologies were modified and adapted to give a distinctive product. CAA (2007) specifies that Reggianito cheese must have a cylindrical shape, be 5–10 kg in weight, low moisture content (<35.9 g/100 g cheese), a minimum fat-in-dry matter of 32 g/100 g dry matter, and a minimum ripening period of 6 months. It is manufactured with pasteurised cow milk and natural whey starter mainly composed of *Lactobacillus helveticus* (66%) and *Lactobacillus delbrueckii* subsp. *lactis* (33%) (Reinheimer, Quiberoni, Tailliez, Binetti, & Suárez, 1996), and generally is ripened at 11–13 °C in 82–85% relative humidity.

Different authors have studied the effect of technological changes during Reggianito Argentino cheese manufacture on ripening (Candioti et al., 2002; Hough, Martinez, Barbieri, Contarini, & Vega, 1994; Hough et al., 1996; Hynes, Bergamini, Suárez, &

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Zalazar, 2003; Lombardi, Bevilacqua, & Califano, 1994; Perotti, Bernal, Meinardi, Candioti, & Zalazar, 2004; Perotti, Bernal, Meinardi, & Zalazar, 2005); however information about Argentinean hard cheeses is relatively scarce. In our research group, different studies related to the effect of elevated temperature on biochemical events and on sensory characteristics during Reggianito Argentino ripening have been carried out (Sihufe, Zorrilla, & Rubiolo, 2009; Sihufe, Zorrilla, Sabbag, Costa, & Rubiolo, 2009; Sihufe et al., 2007). Our objectives were to study the effect of elevated ripening temperature on Reggianito Argentino cheese ripening, considering the physicochemical, proteolysis, lipolysis, and sensory analysis as combined aspects and to determine the optimal ripening time when cheese is ripened at elevated temperatures.

2. Materials and methods

2.1. Cheese sampling and ripening conditions

Data from our preceding papers on Reggianito Argentino cheese (Sihufe et al., 2007; Sihufe, Zorrilla, & Rubiolo, 2009; Sihufe, Zorrilla, & Sabbag et al., 2009) were used in this study for further analysis. Fourteen cheeses (6.9 ± 0.3 kg weight, 23.4 ± 0.4 cm diameter, 12.5 ± 0.4 cm height) manufactured with milk from the same cheese vat were brought from a local factory to our laboratory immediately after brining. Two cheeses were used to determine their initial composition, while the other 12 cheeses were stored at two different temperatures for 6 months. Six cheeses were ripened at 12 °C and 85% relative humidity, and the other six cheeses were ripened at 18 °C and 85% relative humidity. During ripening, cheeses were turned over once a week to maintain uniform composition. Cheeses were analysed in duplicate at 62, 123 and 186 ripening days for the determination of physicochemical, proteolysis, lipolysis and sensory parameters.

2.2. Physicochemical analysis

Samples were completely grated and analysed to determine the moisture and chloride contents (Zorrilla & Rubiolo, 1994), total nitrogen, water-soluble nitrogen at pH 4.6 (Sihufe, Zorrilla, & Rubiolo, 2003), and pH (Sihufe et al., 2007). Fat content was determined for initial composition (IDF, 1969). Index of maturation (*MI*) was expressed as a percentage of water-soluble nitrogen at pH 4.6 of the cheese total nitrogen.

2.3. Proteolysis analysis

Proteolysis during Reggianito Argentino cheese ripening was studied through urea-PAGE of the urea-soluble fraction, RP-HPLC analysis of the water-soluble fraction at pH 4.6 (WSF), and the analysis of free amino acids, as described by Sihufe, Zorrilla, and Rubiolo (2009).

2.4. Lipolysis analysis

Lipolysis during Reggianito Argentino cheese ripening was studied by the analysis of free fatty acids (FFAs), as described by Sihufe et al. (2007).

2.5. Sensory analysis

Sensory characteristics of Reggianito Argentino cheese during ripening were studied using a quantitative descriptive analysis carried out by a panel of eight selected, trained assessors as described by Sihufe, Zorrilla, and Sabbag et al. (2009).

2.6. Statistical analysis

The ripening temperature and ripening time were selected as main factors for ANOVA. When differences between treatment effects were significant ($p < 0.05$), a multiple comparison of means was performed using the least significant differences (LSD) test. Principal component analysis (PCA) was used to reduce the dimensionality of the data studied. It is worth mentioning that in this case, physicochemical, proteolysis and lipolysis data used for further analysis correspond to samples obtained from the central zone of the cheese (Sihufe et al., 2007). Statistical analysis was carried out using Minitab (Minitab Inc., State College, PA).

3. Results and discussion

The most relevant results of our preceding papers (Sihufe et al., 2007; Sihufe, Zorrilla, & Rubiolo, 2009; Sihufe, Zorrilla, Sabbag et al., 2009) are summarised to establish the main characteristics of Reggianito Argentino cheese ripening. This will help to improve the understanding of PCA when all the physicochemical, biochemical and sensorial data are analysed.

3.1. Physicochemical characteristics

Table 1 shows the average values of pH, moisture content, chloride content and maturation index obtained during the ripening of Reggianito Argentino cheese at 12 and 18 °C. Values of pH at the end of ripening were similar to those reported by other researchers for this type of cheese (Hynes et al., 2003; Perotti et al., 2004).

ANOVA showed that ripening temperature and time had significant effects on moisture content. Moisture content values showed a decreasing trend with ripening time, which is typical for cheeses ripened without wrapping and agrees with the information reported by other authors (Simal, Sánchez, Bon, Femenia, & Rosselló, 2001).

When cheeses are salted by brine immersion, salt is taken up and moisture is lost simultaneously. During the ripening stage, salt is redistributed until a uniform concentration is reached throughout the cheese. In the case of Reggianito Argentino cheese, ANOVA showed a significant effect of ripening temperature and time. The value of chloride content at the end of ripening was approximately 1.1 g/100 g cheese. The increase in ripening temperature accelerated the salt redistribution.

The values of *MI* were significantly affected by ripening temperature and time, the values for cheeses stored at 12 °C being similar to those reported by other authors for this cheese (Hynes et al., 2003; Perotti et al., 2004).

Table 1
Average values and standard deviations corresponding to physicochemical parameters determined during Reggianito Argentino cheese ripening at 12 and 18 °C.

Temperature (°C)	Time (days)	pH	Moisture content (g/100 g cheese)	Chloride content (g/100 g cheese)	*MI* (%)
12	62	5.37 ± 0.03	37.5 ± 0.6d	0.51 ± 0.02a	13.8 ± 0.5a
	123	5.47 ± 0.07	36.1 ± 0.4c,d	0.87 ± 0.02c	18.7 ± 1.1b
	186	5.44 ± 0.05	35.2 ± 0.6b,c	1.06 ± 0.01d	19.6 ± 1.3b
18	62	5.47 ± 0.01	36.3 ± 0.3c,d	0.66 ± 0.02b	21.8 ± 0.6c
	123	5.27 ± 0.05	34.6 ± 0.6a,b	1.02 ± 0.02d	22.7 ± 1.8c
	186	5.57 ± 0.07	33.5 ± 0.2a	1.18 ± 0.02e	27.3 ± 1.2d

Average values in the same column with different letters are significantly different ($p < 0.05$).

3.2. Proteolysis

Seven fractions with different electrophoretic mobility were studied (Sihufe, Zorrilla, & Rubiolo, 2009): γ-casein, β-casein, fraction F1, fraction F2, α_{s1}-casein, α_{s1}-I-casein, and fraction F3. Sihufe, Zorrilla, and Sabbag et al. (2009) reported that the most important fractions (α_{s1}- and β-casein) decreased significantly during the first 2 months of ripening, the effect more pronounced being for α_{s1}-casein. This behaviour is common for the majority of cheese varieties, where the activity of the residual coagulant is more important than the plasmin activity (Grappin, Rank, & Olson, 1985; McSweeney & Sousa, 2000; Visser & de Groot-Mostert, 1977). The elevated ripening temperature increased the α_{s1}- and β-casein degradation.

The information obtained from the *MI* was complemented by the RP-HPLC of the WSF. Thirty-six peaks were considered, most of them eluted between 20 and 80 min (Sihufe, Zorrilla, & Rubiolo, 2009). Eleven peaks eluted during the first 30 min of the chromatogram which have hydrophilic characteristics, while eight peaks eluted during the last 25 min of the chromatogram that have hydrophobic characteristics. In general, the increase in ripening temperature or time increased peak areas.

Fifteen amino acids were determined (Sihufe, Zorrilla, & Rubiolo, 2009): Asp, Glu, Asn, Ser, His, Gln, Gly, Ala, Tyr, Met, Val, Phe, Ile, Leu and Lys. The principal free amino acids in all cheese samples were Glu, His, Val, Leu and Lys, amino acid profiles being similar to those previously reported for different cheese varieties (Frau, Massanet, Rosselló, Simal, & Cañellas, 1997; McSweeney & Sousa, 2000). The contents of amino acids in cheeses ripened at 18 °C were higher than at 12 °C. Moreover, the total amino acid amount in cheeses ripened for 123 days at 18 °C were higher than the amount in cheeses ripened for 186 days at 12 °C.

3.3. Lipolysis

Nine FFAs were determined (Sihufe et al., 2007): $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$. Similarly to results reported by Perotti et al. (2005), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acids were the FFAs present at the highest concentration, which is expected because these FFAs are the main components of cow milk triacylglycerides (Lopez, Maillard, Briard-Bion, Camier, & Hannon, 2006; Swaisgood, 1996). Sihufe et al. (2007) reported that the concentration profiles corresponding to the different FFAs analysed for cheeses ripened at 12 °C had an increasing trend until 4 months of ripening, generally remaining without changes during the last 2 months of the period studied. On the other hand, the concentration of the nine FFAs increased in cheeses stored at 18 °C over the 6 months studied, which demonstrates the temperature effect on the lipolytic enzymes present in cheese.

Sihufe et al. (2007) also reported that the total FFA content increases until 4 months of ripening in cheeses stored at 12 °C, remaining almost constant during the last 2 months of ripening. At 18 °C, total FFA content increases over the 6 months evaluated. However, the differences observed between cheeses ripened at different temperatures were significant only for the last 2 months of ripening.

3.4. Sensory analysis

Ten sensory attributes were studied (Sihufe, Zorrilla, & Sabbag et al., 2009): aroma, colour, visual texture, granular cut, fracturability, oral texture, genuine flavour, residual flavour, salty, and bitter. Using PCA, three interesting groupings of sensory attributes were found (Sihufe, Zorrilla, & Sabbag et al., 2009). In the first group

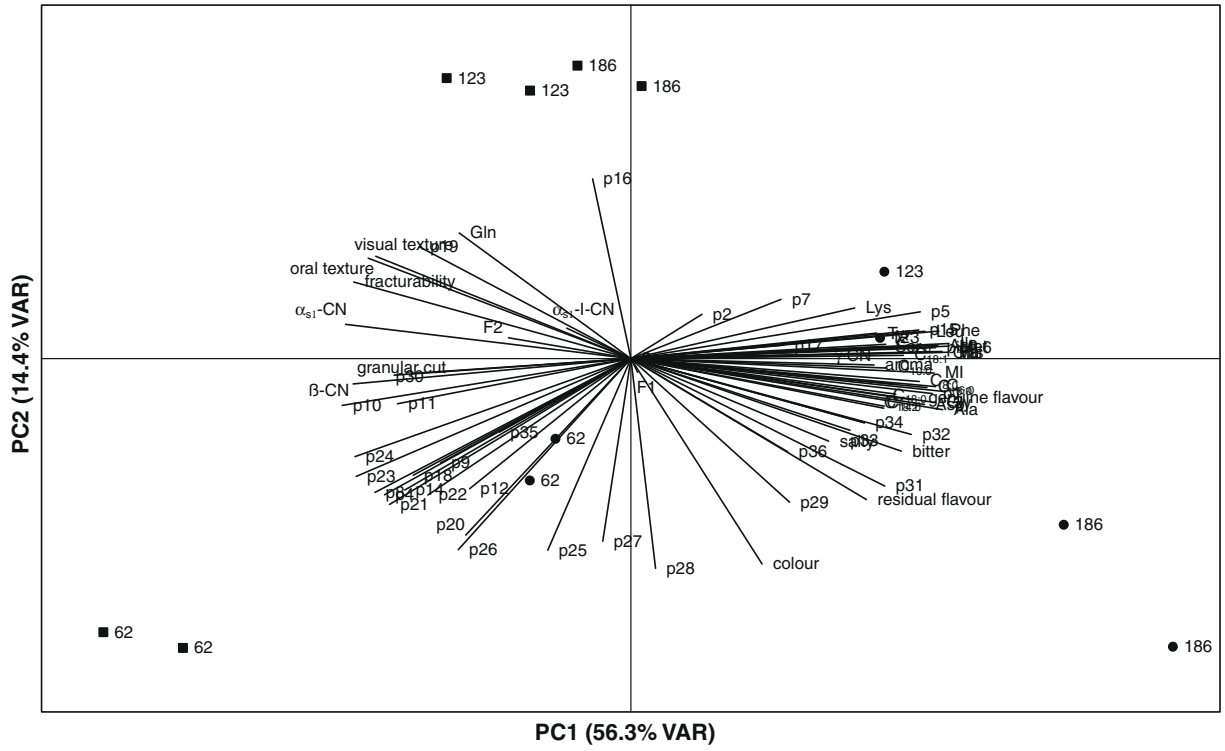

Fig. 1. Biplot of scores and loadings of data obtained from proteolysis, lipolysis and sensory analysis of Reggianito Argentino cheese samples: (■) cheeses ripened at 12 °C; (●) cheeses ripened at 18 °C. Numbers close to symbols indicate days of ripening.

(visual texture, granular cut, fracturability, oral texture), the scores of the attributes remained almost constant in cheeses ripened at 12 °C, while those scores in cheeses ripened at 18 °C showed a clear tendency to decrease with ripening time. In a second group (aroma, genuine flavour, salty), the scores of the attributes in cheeses ripened at 12 °C were slightly lower than those scores in cheeses ripened at 18 °C during the complete ripening period studied. In a third group (colour, residual flavour, bitter), the scores of the

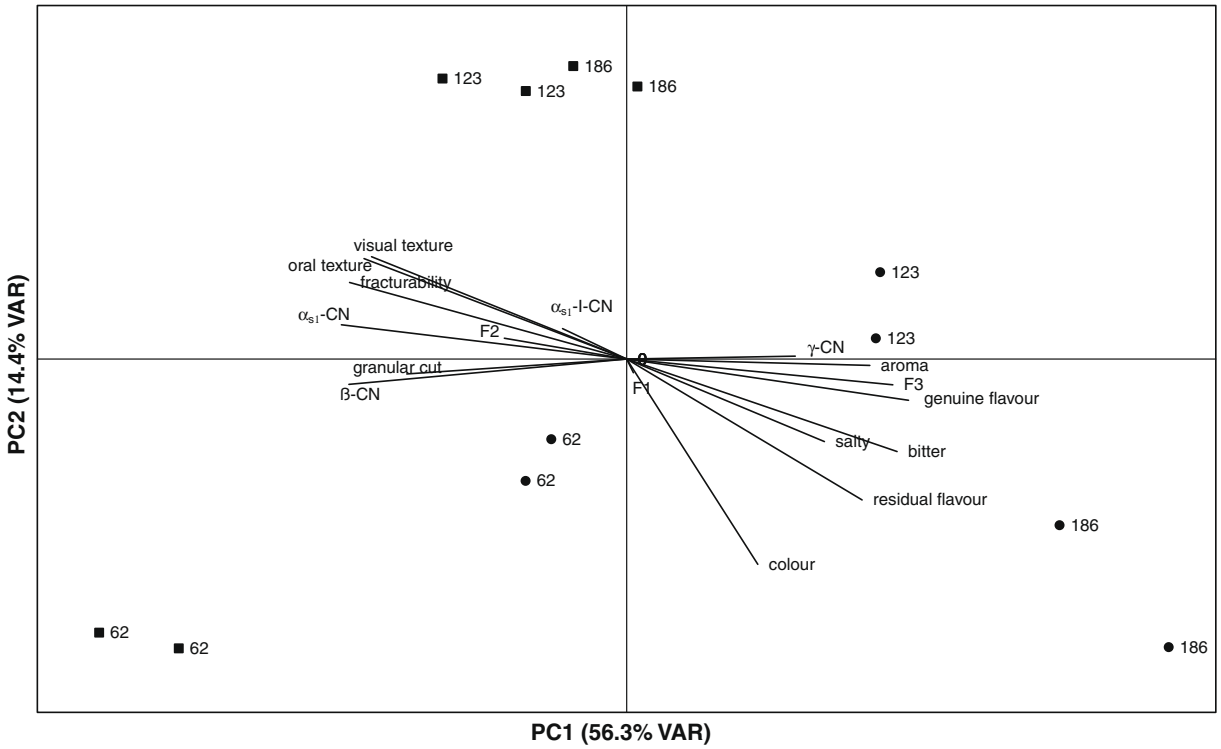

Fig. 2. Biplot of scores and loadings of data obtained from urea-PAGE and sensory analysis of Reggianito Argentino cheese samples: (■) cheeses ripened at 12 °C; (●) cheeses ripened at 18 °C. Numbers close to symbols indicate days of ripening.

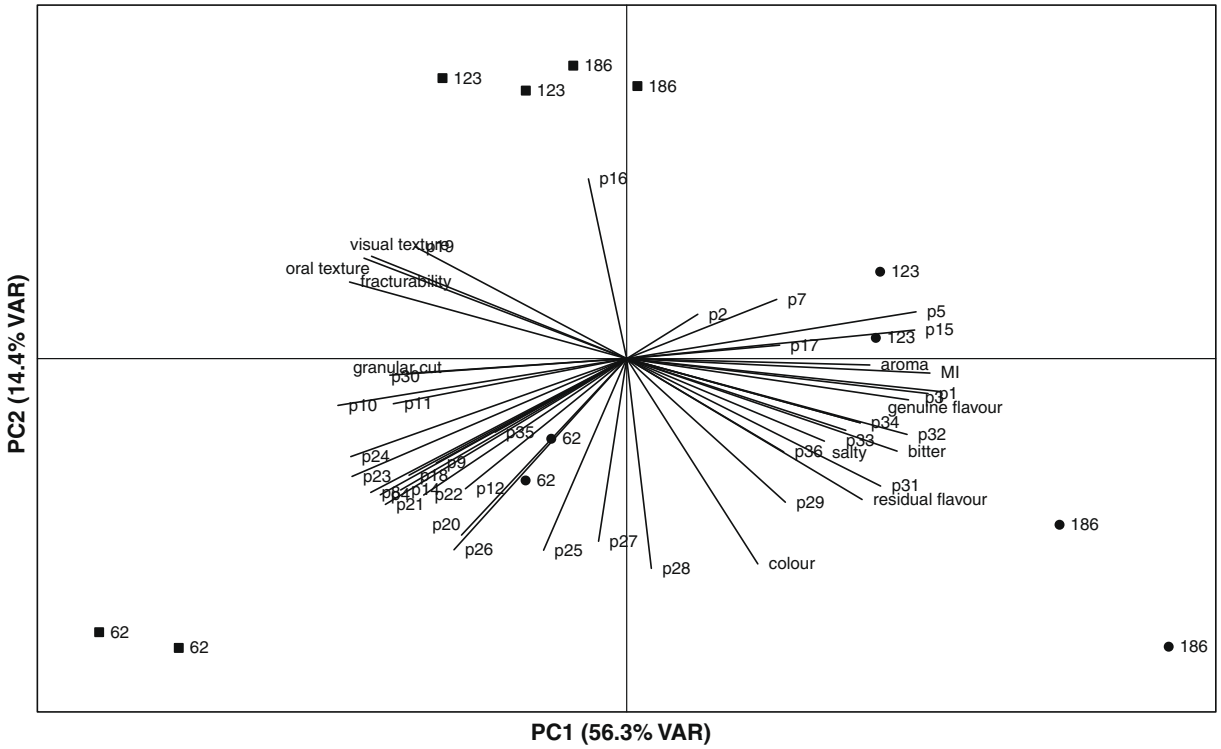

Fig. 3. Biplot of scores and loadings of data obtained from *MI*, RP-HPLC of WSF and sensory analysis of Reggianito Argentino cheese samples: (■) cheeses ripened at 12 °C; (●) cheeses ripened at 18 °C. Numbers close to symbols indicate days of ripening.

attributes remained almost constant in cheeses ripened at 12 °C, while those scores in cheeses ripened at 18 °C showed a clear tendency to increase with ripening time. Although the attribute scores of cheeses ripened at 18 °C were different from those ripened at 12 °C, they did not result in atypical Reggianito sensory characteristics. Moreover, a Reggianito Argentino cheese ripened at 18 °C for 62 days had similar sensory characteristics to a cheese ripened at 12 °C for 186 days.

3.5. Principal component analysis

Two factors were considered for this study: ripening temperature (12 and 18 °C) and ripening time (62, 123 and 186 days). Seventy-eight variables were considered for the PCA: *MI* (%), seven fractions of the urea-PAGE analysis (integrated optical density), 36 peaks of the WSF (peak areas per 100 g cheese), 15 free amino acids (mg amino acid per 100 g cheese), nine FFAs (mg fatty acid per kg cheese), and 10 sensory attributes. As a result, the data set consisted of 12 cheese samples and 78 input variables. Because the input variables differed in magnitude, a correlation matrix was used for PCA.

The biplot of the first two principal components (70.7% variance) is shown in Fig. 1. The first principal component (56.3% variance) was related to ripening time, the second principal component (14.4% variance) being related to the ripening temperature. According to PC1, samples of cheeses ripened 62 days at 18 °C are similar to those cheeses ripened 123 days at 12 °C. However, samples of cheeses ripened 123 days at 18 °C were not similar to those ripened 186 days at 12 °C. Therefore, the optimal time to ripen cheeses at 18 °C may be between 2 and 3 months.

Some interesting groupings between measured variables and samples were observed (Figs. 2–5). To improve the clarity of presentation of the results, the biplot is shown considering the sensory variables combined with the variables obtained through urea-PAGE, RP-HPLC of WSF, free amino acids, and FFA analysis. Clearly, the decrease of the concentration of the main urea-PAGE fractions (α_{s1}- and β-casein) is related to the changes observed in the attributes related to cheese texture (visual and oral texture, fracturability, and granular cut) (Fig. 2).

Samples of cheeses ripened 186 days at 18 °C can be related to bitter and residual flavour development. Moreover, some peaks corresponding to the hydrophobic region of the chromatogram (p31, p32, p33, p34 and p36) may be related to bitterness (Engel, Nicklaus, Septier, Salles, & Le Quéré, 2001; McSweeney & Sousa, 2000) (Fig. 3).

Samples of cheeses ripened 123 days at 18 °C can be related to aroma and genuine flavour development. The maturation index, some peaks corresponding to the hydrophilic region of the chromatogram, the free amino acids, and the FFAs may be related to those attributes (Figs. 4 and 5). These results reinforce the idea that some amino acids, such as Glu or Leu, may contribute to the development of Reggianito cheese flavour, taking into account their flavour-enhancing properties (Glu) or their importance as a precursor of branched-chain volatile flavour compounds (Leu) (Fallico et al., 2004; Sihufe, Zorrilla, & Rubiolo, 2009). Moreover, it is usually considered that short and intermediate-chain FFAs have a significant impact on the development of characteristic cheese flavour (Collins, McSweeney, & Wilkinson, 2003; Georgala et al., 2005; Lopez et al., 2006) and indeed in this case were related to flavour development.

Variables with the most important PC1 loadings were analysed to determine the time when samples ripened at 18 °C have similar values to samples ripened 6 months at 12 °C. The optimal time for ripening Reggianito Argentino cheese at 18 °C ranged between 2 and 3 months. It is worth mentioning that the acceleration due to the elevated temperature in the lipolysis changes was slower than for the proteolysis changes. Moreover, the ripening of Reggianito Argentino cheese at 18 °C for 4 months may contribute

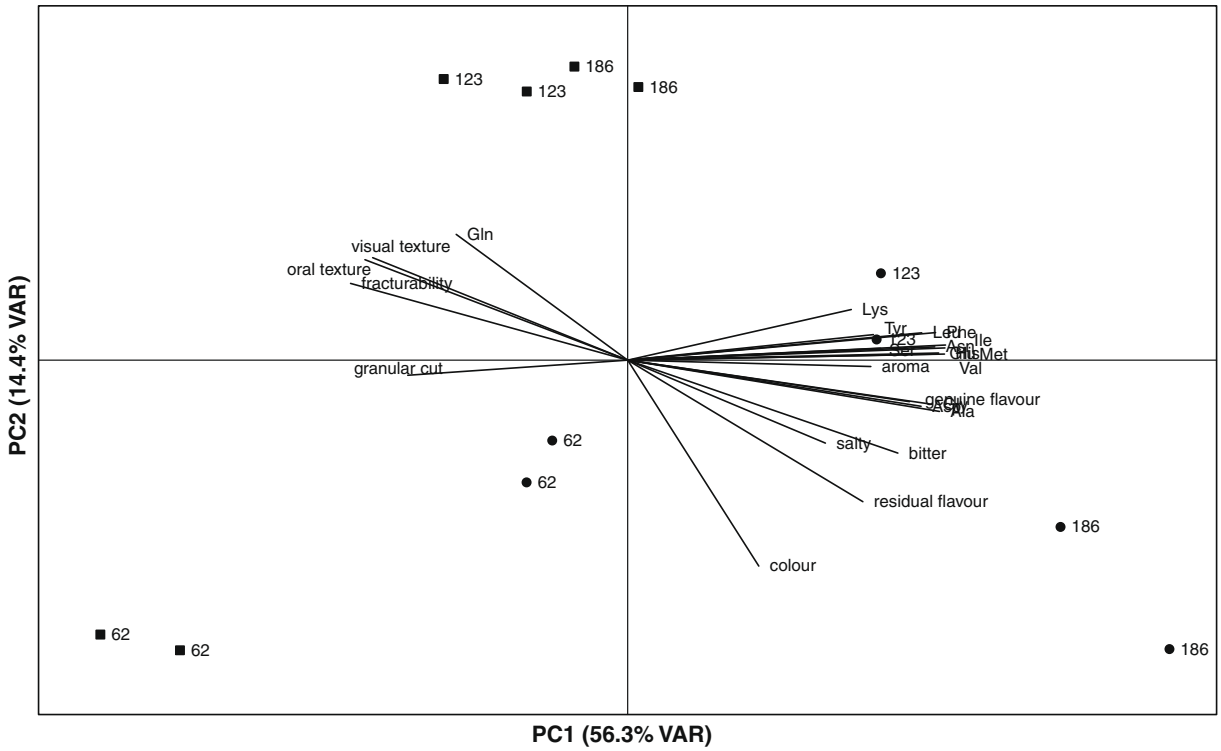

Fig. 4. Biplot of scores and loadings of data obtained from free amino acids and sensory analysis of Reggianito Argentino cheese samples: (■) cheeses ripened at 12 °C; (●) cheeses ripened at 18 °C. Numbers close to symbols indicate days of ripening.

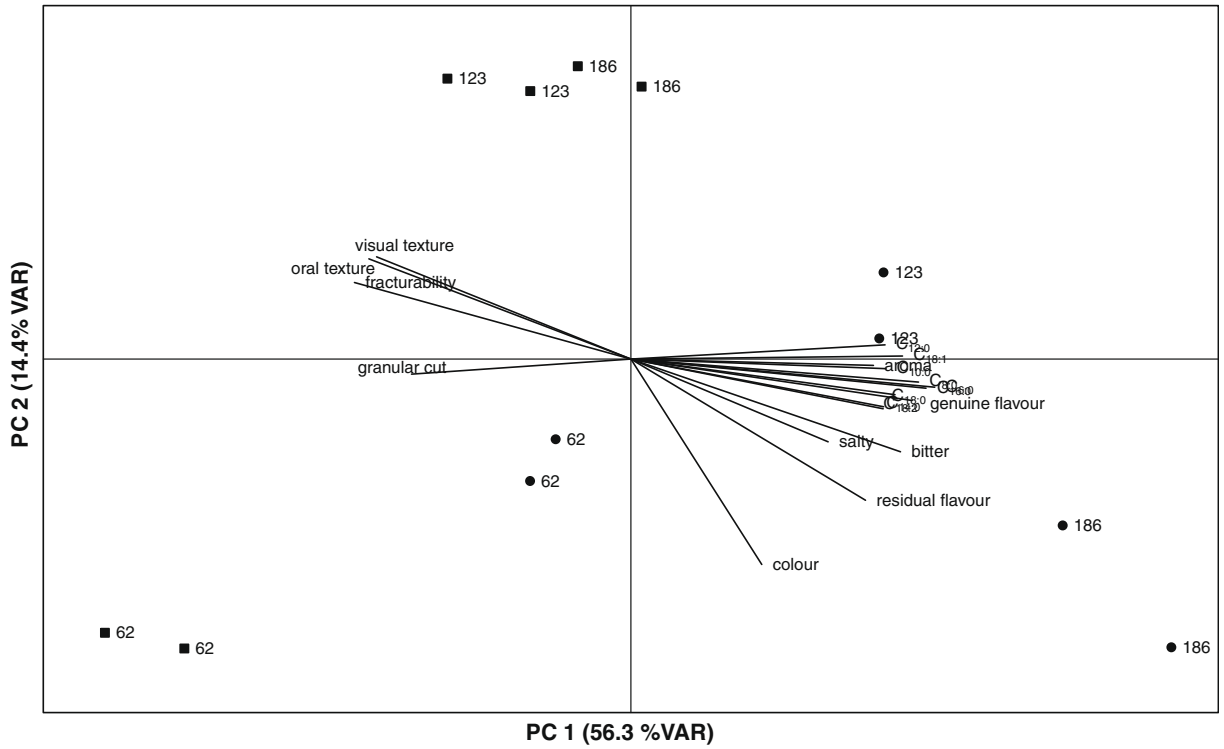

Fig. 5. Biplot of scores and loadings of data obtained from free fatty acids and sensory analysis of Reggianito Argentino cheese samples: (■) cheeses ripened at 12 °C; (●) cheeses ripened at 18 °C. Numbers close to symbols indicate days of ripening.

favourably to the development of aroma and genuine flavour of cheeses. Finally, it is worth recalling that when elevated ripening temperatures are used, it is highly recommended to ensure good manufacturing conditions to reduce the risk of the growth of unwanted microbial contaminants (Azarnia et al., 2006).

4. Conclusions

The influence of elevated temperature on Reggianito Argentino cheese ripening was evaluated. Principal component analysis conveniently summarised the information obtained from physicochemical, proteolysis, lipolysis, and sensory analyses. The first principal component accounted for 56.3% of the total variance and can be related to the ripening time, while the PC2 (14.4% variance) can be related to the ripening temperature. Useful groupings were visualised by biplot of the first two principal components. The optimal time for ripening Reggianito Argentino cheese at 18 °C ranged between 2 and 3 months. However, it is relevant to point out that when elevated temperature is used during cheese ripening, very hygienic conditions should be ensured.

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