# ORIGINAL RESEARCH

# Effect of increased initial ripening temperature on the sensory characteristics of Reggianito cheese

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The effects of increased initial ripening temperature on the sensory characteristics of Reggianito cheese were evaluated in relation to different temperature–time combinations. Control cheeses stored at 12 °C for 6 months and experimental cheeses stored at 20 °C for 2 or 4 weeks then at 12 °C up to 6 months were analysed at 61, 124, and 180 days of ripening by physicochemical, microbiological and quantitative descriptive analysis. The use of an initial increased ripening temperature results in controlled and limited changes on mesophilic lactobacilli growth and in a mild acceleration of the sensory characteristic development of Reggianito cheese.

Keywords Reggianito, Cheese, Sensory characteristics, Ripening, Initial increased temperature.

#### INTRODUCTION

According to the American Society for Testing and Materials, sensory evaluation can be defined as 'a scientific discipline used to evoke, measure, analyse and interpret reactions to the characteristics of foods and materials as they are perceived by the senses of sight, taste, touch and hearing', a definition that can be paraphrased as 'a set of tools to accurately measure human responses to external stimuli' (Drake and Delahunty 2011). A diversity of compounds with a major impact on flavour are produced together with textural and appearance changes as a consequence of the several biochemical transformations occurring in cheese during ripening. As long as technological changes and innovations during the manufacture of cheese can impact on these biochemical pathways, their effects on sensory characteristics must be investigated by sensory analysis, as feasibility of these changes is subjected to acceptance among consumers.

Several attempts have been made to accelerate cheese ripening, because this is a long and thus expensive step of cheese manufacturing, particularly in low moisture, slow-ripening varieties (Folkertsma *et al.* 1996). Among the currently

available alternatives to shorten cheese storage time are the following: the direct addition of exogenous enzymes, the incorporation of attenuated starters and adjunct cultures, the use of startmodified by genetic engineering, the ers microencapsulation of ripening enzymes and the elevation of ripening temperature (Azarnia et al. 2006). Increasing storage temperature stands out as being a simple and inexpensive alternative that can represent additional benefits resulting from lower refrigeration costs (O'Mahony et al. 2006). Another advantage associated with this technology is that no external agents are added to the cheese mass so overcoming potential restrictions set by product regulations, as Protected Denomination of Origin (P.D.O.; Ferrazza et al. 2004). Main drawbacks concerning this technology are an increased risk of the development of undesirable micro-organisms, as well as nonspecific increases in ripening reactions with a negative impact on sensory characteristics, such as the development of unbalanced flavours, off-flavours and textural defects (e.g. softening or crumbliness; Law 2001). In particular, the application of high storage temperatures during the initial stages of ripening seems to be an effective strategy to obtain an increase in the activity of enzymatic

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© 2014 Society of Dairy Technology systems, whereas it seems to provide a reduction in the risk of microbiologic contamination or unbalance in biochemical reactions (Aston *et al.* 1983; Ferrazza *et al.* 2004; Hannon *et al.* 2005; O'Mahony *et al.* 2006).

Reggianito cheese is the most important hard cheese variety produced in Argentina, widely consumed locally and exported to several countries worldwide. It was developed in the late 19th and early 20th centuries, as an adaptation of cheesemaking technologies for hard Italian cheeses used by Italian immigrants. Pasteurised cow's milk is used for the cheese manufacture (Sihufe *et al.* 2012), and natural whey culture composed of thermophilic lactobacilli such as *Lactobacillus helveticus* (66%) and *Lactobacillus delbrueckii* subsp. *lactis* (33%; Reinheimer *et al.* 1996) is used as starter. Reggianito cheese is generally ripened at 11–13 °C and 82–85% relative humidity. According to CAA (2006), Reggianito cheese must have a cylindrical shape, with 5–10 kg weight, low moisture content (<35.9 g/100 g cheese) and a minimum ripening time of 6 months.

Previous research has been carried out to elucidate the effects of technological changes applied during Reggianito cheese manufacturing (Hough et al. 1994; Candioti et al. 2002; Hynes et al. 2003; Perotti et al. 2005). In relation to accelerated ripening of this cheese, different studies have been carried out to assess the impact of an increased storage temperature to 18 °C for 6 months on the lipolysis (Sihufe et al. 2007), proteolysis (Sihufe et al. 2010a) and sensory characteristics (Sihufe et al. 2010b), as well as a statistical analysis taking into account all that information (Sihufe et al. 2010c). In addition to characterising the main transformations occurring during ripening, those studies allowed an optimal ripening period between 2 and 3 months to be established when cheeses are stored to 18 °C. To accelerate Reggianito cheese ripening, it seems possible to apply higher initial storage temperatures instead of treatments based on ripening at high temperatures during the whole ripening period. Aimed at this goal, the effect of different temperature-time combinations on proteolysis during ripening of Reggianito cheese had been previously evaluated (Ceruti et al. 2012). Information relating to other ripening areas was needed to obtain for a general view of the impact of those changes in ripening regime. Undoubtedly, flavour, texture and/or appearance of cheeses are essential characteristics that must be assessed to guarantee the success of the treatments proposed to accelerate cheese ripening. In the present study, our objective was to evaluate the effects of different temperature-time combinations during ripening on the sensory characteristics of Reggianito cheese.

# MATERIALS AND METHODS

#### Cheese sampling and ripening conditions

Twenty cheeses (7.8  $\pm$  0.1 kg weight, 23.7  $\pm$  0.2 cm diameter, 15.3  $\pm$  0.2 cm height) were manufactured at a local

factory with milk from the same cheese vat and by a standard cheesemaking procedure and were brought to our laboratory after 7 days of brining. Two cheeses were used to determine initial composition, while the other 18 cheeses were stored for 6 months at three different temperature–time combinations. Six cheeses were ripened at 12 °C and 85% relative humidity (cheeses C), six cheeses were ripened at 85% relative humidity and 20 °C for 2 weeks followed by 12 °C up to 6 months (cheeses E<sub>1</sub>) and six cheeses were ripened at 85% relative humidity and 20 °C for 4 weeks followed by 12 °C up to 6 months (cheeses E<sub>2</sub>). Cheese analysis was performed in duplicate at 61, 124 and 180 days of ripening.

## Physicochemical analysis

Samples were grated and analysed to determine the moisture and chloride contents. Moisture content was measured using a microwave oven CEM AVC 80 (CEM, Matthews, NC, USA), while chloride content was quantified following a potentiometric method using an automatic titrator model DL40RC (Mettler Instrumente AG, Greifensee, Switzerland; Zorrilla and Rubiolo 1994). For pH determination, a pH electrode for solid foods was used (pH Spear; Oakton Instruments, Vernon Hills, IL, USA). The initial composition of the fat content estimated by a gravimetric method (IDF 1969) and total nitrogen content determined by the micro-Kjeldahl method as described in Sihufe *et al.* (2003). Determinations were carried out in duplicate; the chloride content was determined in triplicate.

# Microbiological analysis

Basic knowledge of microbial flora during cheese ripening is of prime importance for ensuring final cheese quality. Cheese samples of 10 g obtained from central zone at 0, 61, 124 and 180 days of ripening were suspended in a 90 mL sterile 2% (w/v) sodium citrate solution and then homogenised in sterility to give a 1/10 dilution. Three serial decimal dilutions, prepared by mixing 10 mL with 90 mL of 0.1% (w/v) sterile peptone water, were then plated on MRS agar (Biokar, Beauvais, France) and incubated for 3 days at 30 and 42 °C. A temperature of 42 °C was selected to promote the growth of the starter flora that consists of thermophilic lactobacilli (Reinheimer et al. 1996). A lower incubation temperature of 30 °C was chosen to obtain tentative counts of nonstarter lactic acid bacteria (NSLAB), mainly consisting of mesophilic lactobacilli (Bude Ugarte et al. 2006). Microscopical observation of the colonies grown on the plates was made to corroborate cell morphology. After incubation, colonies were counted and the results expressed as a logarithm of colony-forming units Log (cfu/g cheese).

#### Sensory analysis

The quality of cheese is determined by its flavour and texture attributes. Particularly in the case of cheese ripening

acceleration, sensory analysis is a key activity to detect whether the method proposed results in a controlled and balanced flavour quality. Quantitative descriptive sensory analysis of cheese samples was carried out using 10 sensory attributes that were evaluated by a trained seven-assessors panel (Table 1). Panel members had previous experience working with samples of Reggianito cheese and developing the terminology used in this work (Candioti et al. 2002; Sihufe et al. 2010b). Before sensory analysis, panel members were trained using local commercial samples of Reggianito cheese to re-acquaint them with terms used to describe this cheese. Each sensory attribute was evaluated twice by each assessor. Cheeses were sampled at 61, 124 and 180 days of ripening, and sensory evaluation was made the same day that cheese samples were obtained. Before each evaluation session, panel members took part of a calibration session, using Reggianito cheese samples from local market in order to agree on terms and scales. Samples were placed on a circular sector of 10 cm radius, 1 cm arc length and 1 cm height approximately and were cut avoiding the area close to the rind. Samples identified by random numbers were presented in a randomised order and evaluated on individual trays. Sensory attributes were scored by marking on an unstructured 10-cm scale anchored at each end. Sensory assessment was conducted in individual booths at a sensory laboratory, which complies with international standards for the design of test rooms. Free access to water and unsalted crackers was provided to each assessor for palate cleansing between samples. Scores for each sample were averaged over all assessors and replicates.

#### Statistical analysis

For statistical analysis, ripening conditions and ripening time were selected as main factors for ANOVA, performed

 Table 1 Sensory attributes used for Reggianito cheese (Sihufe et al. 2010b)

Attribute	Definition
Aroma	Perceived total intensity of aroma
Colour	The colour of cheese
Visual texture	Number of openness, number of cracks, etc.
Granular cut	Perception of moderate sized particles in the cheese surface after rupture <sup>a</sup>
Fracturability	Degree of rupture <sup>a</sup>
Oral texture	Roughness, sandy, pasty <sup>b</sup>
Genuine flavour	Perceived intensity of flavour
Residual flavour	Residual pleasant or unpleasant flavour
Salty	Salty taste
Bitter	Bitter taste

<sup>a</sup>Samples were bent until rupture with forefingers and thumbs of both hands. <sup>b</sup>Samples were evaluated after 4–5 chews; samples were expectorated.

using Statgraphics (Statgraphics Inc., Rockville, MD, USA). For significant differences (P < 0.05) between treatment effects, a multiple comparison of means was performed using Least Significant Differences (LSD) test. Principal Component Analysis (PCA) was applied to reduce the dimensionality of sensory analysis data to a smaller subset. The analysis was carried out using Minitab (Minitab Inc., State College, PA, USA).

# **RESULTS AND DISCUSSION**

## **Physicochemical characteristics**

The initial composition of cheeses was  $40.1 \pm 0.2\%$  (w/w) moisture,  $20.8 \pm 1.5\%$  (w/w) fat,  $33.1 \pm 0.3\%$  (w/w) protein, nondetectable chloride, and the pH was  $5.24 \pm 0.02$ . Table 2 shows the average values for pH, moisture content, and NaCl content obtained during ripening of Reggianito cheese for the treatments studied. The values obtained are consistent with values previously reported for this type of cheese as discussed by Ceruti *et al.* (2012).

# Microbiological characteristics

Microscopical observation confirmed cell morphology corresponding to lactobacilli in cultures obtained after cheese sampling and grown at 30 and 42 °C. As mentioned above, Reggianito cheese is manufactured using natural whey starters mainly composed by *L. helveticus* and *L. delbrueckii* subsp. *lactis* (Reinheimer *et al.* 1996). Cultures at 42 °C showed values of  $10^{6}$ – $10^{7}$  cfu/g after cheese brining, starter counts being nondetectable (< $10^{3}$  cfu/g) from 61 days of ripening onwards. Accordingly, Coppola *et al.* (2000) and Giraffa *et al.* (1997) have reported that thermophilic lactobacilli decreased progressibly within early ripening stages in hard Italian cheeses using natural whey cultures as starter (Parmigiano Reggiano and Grana Padano).

For cultures grown at 30 °C, bacterial counts for cheeses at the beginning of ripening were  $1.9 \pm 0.4 \times 10^7$  cfu/g. Values for the different temperature-time combinations at 61, 124 and 180 days of ripening are shown in Table 3. According to ANOVA, plate counts corresponding to NSLAB were significantly affected by both ripening time and timetemperature combinations. From 61 days of ripening onwards, NSLAB cell number showed a slight decrease over time for all treatments. Values observed for experimental cheeses  $E_1$  and  $E_2$  were similar during ripening, being both one log order below those for control cheeses from 124 days of ripening onwards. These results suggest that only controlled and limited changes were observed on mesophilic lactobacilli growth when the initial storage temperature was increased during ripening. Moreover, pH values obtained reinforced the idea of a controlled ripening acceleration from the microbiological point of view (Table 2), due to pH values within a range considered safe in relation to potential spoilage with unusual micro-organisms.

Cheese	Time (days)	pН	Moisture (g/100 g cheese)	NaCl (g/100 g cheese)
С	61	$5.54 \pm 0.01^{\rm cd}$	$37.9 \pm 0.0^{a}$	$0.91 \pm 0.06^{a}$
	124	$5.36 \pm 0.03^{a}$	$36.9 \pm 0.1^{\circ}$	$1.45 \pm 0.02^{\circ}$
	180	$5.70 \pm 0.02^{\rm ef}$	$36.1 \pm 0.4^{e}$	$1.71\pm0.01^{ m d}$
$E_1$	61	$5.56 \pm 0.04^{\rm d}$	$37.7 \pm 0.1^{ab}$	$0.97\pm0.03^{ m a}$
	124	$5.41 \pm 0.01^{\rm ab}$	$36.7 \pm 0.5^{\rm cd}$	$1.52\pm0.08^{ m c}$
	180	$5.76 \pm 0.02^{\rm fg}$	$35.8 \pm 0.1^{e}$	$1.79\pm0.02^{ m d}$
E <sub>2</sub>	61	$5.60 \pm 0.10^{de}$	$37.2 \pm 0.1^{\rm bc}$	$1.16 \pm 0.03^{\rm b}$
	124	$5.45 \pm 0.01^{\rm bc}$	$36.3 \pm 0.2^{de}$	$1.52\pm0.01^{ m c}$
	180	$5.83 \pm 0.02^{ m g}$	$35.9 \pm 0.0^{\rm e}$	$1.75\pm0.00^{ m d}$
Ripening condition		*	*	*
Time		*	*	*
Interaction		NS	NS	*

Table 2 Average values and standard deviation corresponding to physicochemical parameters determined during Reggianito cheese ripening

Last rows show the ANOVA result for the different factors analysed. Average values in the same column with different superscript letters are significantly different (P < 0.05). \*Significant effect (P < 0.05). NS, No significant effect (P > 0.05).

 Table 3 Average and standard deviation of microbial counts corresponding to MRS plates at 30 °C, determined during Reggianito cheese ripening

Cheese	Time (days)	Log cfu/g
С	61	$8.50 \pm 0.20^{ m g}$
	124	$7.70 \pm 0.10^{\rm ef}$
	180	$7.20 \pm 0.30^{cde}$
E1	61	$8.02\pm0.03^{ m fg}$
	124	$6.73 \pm 0.01^{bcd}$
	180	$5.80\pm0.90^{ m a}$
E <sub>2</sub>	61	$7.50\pm0.20^{\rm def}$
	124	$6.51\pm0.05^{abc}$
	180	$6.10\pm0.40^{ m ab}$
Ripening conditio	n	*
Time		*
Interaction		NS

Last rows show the ANOVA result for the different factors analysed. Average values in the same column with different superscript letters are significantly different (P < 0.05). \*Significant effect (P < 0.05). NS, No significant effect (P > 0.05).

#### Sensory analysis

Table 4 shows the average values of the 10 sensory attributes studied for the different treatments applied during Reggianito cheese ripening. The scores of the attributes for cheeses ripened at 12 °C for 180 days are similar to those for cheeses ripened under the same conditions reported by Candioti *et al.* (2002) and Sihufe *et al.* (2010b). Comparing the evolution of the 10 sensory attributes evaluated for control cheeses from 61 to 180 days of ripening, it can be concluded that genuine flavour and salty taste were the attributes changing significantly and therefore representing the

difference between a fully ripened 180-days Reggianito cheese and an immature 61-days one.

The increase of the genuine flavour and salty taste attributes with ripening time can be addressed in relation to some of the different changes that occur during cheese ripening. It is known that several biochemical pathways take place during ripening, for example, catabolism of lactate and citrate, lipolysis and proteolysis, which all contribute to cheese flavour development (McSweeney 1997). Particularly, a clear association of aroma and genuine flavour development with the production of free amino acids and free fatty acids was found during Reggianito cheese ripening (Sihufe et al. 2010c). Indeed, Ceruti et al. (2012) found that the total amino acid content at 180 days of ripening was approximately twice the content at 61 days of ripening. In the case of the salty taste, it is worth recalling that salt diffusion in cheeses salted by brine immersion is a slow process in which the salt content gradually increases in inner zones and decreases on the more external close-to-rind zones until a uniform salt concentration is reached (Sihufe et al. 2007). Moreover, NaCl concentrations obtained can clearly be related to this behaviour (Table 2).

No significant effect (P > 0.05) of the interaction between temperature–time combination and ripening time factors was observed (Table 4). The time-temperature combination only affected the attributes aroma and bitter taste. Therefore, it can be inferred that the use of an initial increased ripening temperature results in mild changes in the characteristic sensory development of Reggianito cheese. Sihufe *et al.* (2010b) studied the influence of increased ripening temperature (18 °C) during the whole ripening period on sensory characteristics of Reggianito cheese. Those authors found that the ripening temperature affected all the sensory attributes. In particular, the scores

											Residual
Cheese	Cheese Time (days) Aroma	Aroma	Colour	Visual texture	Granular cut	Granular cut Fracturability	Oral texture	Genuine flavour Salty	Salty	Bitter	flavour
C	61	$4.34\pm0.07^{\mathrm{ab}}$	$5.1\pm0.1^{ m bc}$	$8.083 \pm 0.004^{a}$	$7.8\pm0.2^{ m bc}$	$8.64 \pm 0.04^{\mathrm{bcd}}$	$7.6\pm0.2^{\mathrm{a}}$	$4.4 \pm 0.4^{a}$	$4.44 \pm 0.03^{a}$	$2.42\pm0.06^{a}$	$2.7\pm0.3^{a}$
	124	$4.0\pm0.4^{\mathrm{a}}$	$4.4\pm0.3^{\mathrm{a}}$	$8.06\pm0.04^{\rm a}$	$8.0\pm0.1^{ m cd}$	$8.8\pm0.1^{\mathrm{e}}$	$7.6\pm0.4^a$	$5.6\pm0.5^{ m ab}$	$4.38 \pm 0.01^{\rm a}$	$2.6\pm0.6^{ m ab}$	$3.32 \pm 0.01^{a}$
	180	$4.1\pm0.2^{\mathrm{ab}}$	$5.3\pm0.2^{ m bc}$	$8.08\pm0.02^{\rm a}$	$7.5\pm0.3^{\mathrm{ab}}$	$8.56\pm0.01^{\rm ab}$	$7.8\pm0.4^a$	$5.7\pm0.2^{ m b}$	$5.2\pm0.1^{ m bc}$	$3.0\pm0.2^{ m bc}$	$2.5\pm0.4^{a}$
E1	61	$5.1\pm0.9^{ m ab}$	$5.5\pm0.2^{ m c}$	$8.0\pm0.1^{\rm a}$	$7.6\pm0.1^{ m ab}$	$8.6 \pm 0.2^{ m bcde}$	$7.6\pm0.2^{\rm a}$	$5.1\pm0.3^{ m ab}$	$4.6\pm0.7^{\mathrm{a}}$	$2.82\pm0.01^{\rm abc}$	$2.9\pm0.2^{\mathrm{a}}$
	124	$4.7 \pm 1.4^{ m ab}$	$4.8\pm0.7^{\mathrm{ab}}$	$7.98\pm0.02^{\rm a}$	$8.1\pm0.1^{ m cd}$	$8.81\pm0.04^{\rm de}$	$7.5\pm0.2^{\mathrm{a}}$	$5.6\pm1.3^{ m ab}$	$4.7\pm0.1^{ m ab}$	$3.0\pm0.2^{ m bc}$	$3.4\pm0.3^{\mathrm{a}}$
	180	$5.2~\pm~1.0^{ m ab}$	$4.8\pm0.2^{\mathrm{ab}}$	$7.9\pm0.2^{\mathrm{a}}$	$7.5\pm0.2^{\mathrm{ab}}$	$8.60\pm0.01^{\rm abc}$	$7.0\pm0.4^{\mathrm{a}}$	$6.1\pm0.4^{ m b}$	$5.4\pm0.1^{ m c}$	$3.08\pm0.03^{ m bc}$	$3.2\pm0.6^{\mathrm{a}}$
$E_2$	61	$5.5\pm0.5^{ m ab}$	$5.4\pm0.1^{ m bc}$	$8.0\pm0.1^{\rm a}$	$7.8\pm0.2^{ m bc}$	$8.6\pm0.2^{\mathrm{ab}}$	$7.6\pm0.2^{\rm a}$	$5.1\pm0.5^{ m ab}$	$4.5\pm0.5^{\mathrm{a}}$	$2.9\pm0.1^{ m bc}$	$3.1\pm0.1^{\mathrm{a}}$
	124	$5.4\pm0.7^{ m ab}$	$5.3\pm0.1^{ m bc}$	$8.0\pm0.1^{\rm a}$	$8.3\pm0.1^{\rm d}$	$8.80\pm0.05^{cde}$	$7.53 \pm 0.01^{a}$	$5.9\pm0.1^{ m b}$	$5.22\pm0.02^{\mathrm{bc}}$	$3.0\pm0.1^{ m bc}$	$2.7\pm0.1^{\rm a}$
	180	$5.7\pm0.1^{ m b}$	$4.9\pm0.1^{ m abc}$	$7.9\pm0.04^{\mathrm{a}}$	$7.3\pm0.3^{\mathrm{a}}$	$8.4\pm0.1^{\rm a}$	$7.2\pm0.2^{a}$	$5.9\pm0.4^{ m b}$	$5.5\pm0.2^{ m c}$	$3.2\pm0.1^{ m c}$	$2.8\pm0.5^{\rm a}$
Ripening	Ripening condition	*	NS	NS	NS	NS	NS	NS	NS	*	NS
Time		NS	*	NS	*	*	NS	*	*	NS	NS
Interaction	on	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

of visual texture, granular cut, fracturability and oral texture in cheeses ripened at 18 °C showed a clear tendency to decrease with ripening time, while the scores of colour, bitter taste and residual flavour in cheeses ripened at 18 °C showed the opposite tendency. Although the attributed scores corresponded to typical Reggianito sensory characteristics (Sihufe et al. 2012), it is preferable to have behaviour patterns similar to those of the control cheeses. In our case, scores corresponding to experimental cheeses at 61 and 124 days of ripening were closer to scores corresponding to fully ripened control cheeses (Figure 1). Moreover, Ceruti et al. (2012) had previously studied the proteolysis of Reggianito cheese as affected by the initial elevation of ripening temperature. This had shown that the concentrations of major caseins decreased while the levels of peptides and amino acids increased at higher rates in experimental cheeses although following a similar pattern in control cheeses, so reinforcing the idea of a controlled acceleration of Reggianito cheese maturation.

Using PCA, the information relating to the sensory analysis of cheeses was summarised in a biplot graph (Figure 2). This multivariate methodology retains variables that contribute significantly to the main components and the effect of ripening conditions on the attributes of the cheese that can be determined more objectively. The two first principal components PC1 and PC2 accounted for 35.8% and 22.2% of the total variation. As PCA score values spread from right to left along PC1 from 61 to 180 days of ripening, it can be inferred that this first principal component may be related to ripening time, whereas no clear association of any factor within the distribution of PCA scores along PC2 axis could be traced. Taking into account the relative position of PCA scores with respect to the PC1 axis, it can be suggested that experimental cheeses at 61 and 124 days of ripening had an equivalent ripening time to control cheeses at 180 days of ripening. The distribution of the PCA-loadings along the PC1-axis show that attributes related to cheese texture (visual texture, granular cut, fracturability and oral texture) characterise young cheeses, while attributes related to cheese flavour (aroma, genuine flavour, salty taste and bitter taste) are characteristic of aged cheeses.

The importance of storage temperature on the ripening of Reggianito cheese can therefore be regarded as a relevant result. In our case, a difference of 8 °C from the traditional ripening temperature or 2 °C from the increased temperature selected in previous studies (Sihufe *et al.* 2010b), certainly introduced detectable differences in flavour and texture attributes. The complexity of biochemical pathways occurring during the ripening of Reggianito cheese are sensitively affected by temperature. Therefore, if the acceleration of Reggianito cheese ripening by elevation of storage temperature is the goal, special attention to the temperature history must be taken into account.

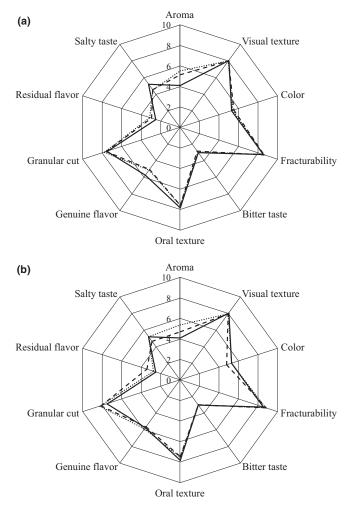


Figure 1 Comparison of mean values for the 10 sensory attributes assessed between control cheeses at 180 days of ripening (—) and experimental cheeses,  $E_1$  (- -) and  $E_2$  (...), at (a) 61 days of ripening and (b) 124 days of ripening.

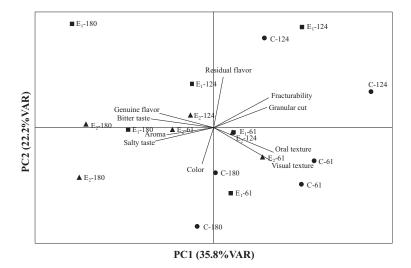


Figure 2 Biplot of scores and loadings of data corresponding to sensory analysis of Reggianito cheese ripened under different temperature-time combinations. Numbers indicate days of ripening of samples.

# CONCLUSIONS

The use of initial increased ripening temperatures was studied as an alternative to accelerated Reggianito cheese maturation. A moderately increased temperature of 20 °C (8 °C higher than the traditional ripening temperature for Reggianito cheese) was used for 2 or 4 weeks at the beginning of maturation in experimental cheeses. Counts for cultures grown at 30 °C remained consistently within acceptable levels for both control and experimental cheeses, while counts for cultures at 42 °C for all samples decreased to nondetectable levels within the first 2 months of ripening. The time-temperature combination only significantly affected aroma and bitter taste among the 10 sensory attributes studied. A mild acceleration of the sensory characteristic development of Reggianito cheese was observed. Taking into account the sensory characteristics studied, experimental cheeses at 61 and 124 days of ripening had equivalent characteristics to control cheeses at 180 days of ripening. These results complement other areas of the ripening biochemistry of Reggianito cheese in the determination of an adequate time-temperature combination and accelerated maturation.

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#### REFERENCES

- Aston J W, Fedrick I A, Durward I G and Dulley J R (1983) The effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese I: higher initial storage temperatures. *New Zealand Journal of Dairy Science and Technology* 18 143–151.
- Azarnia S, Robert N and Lee B (2006) Biotechnological methods to accelerate Cheddar cheese ripening. *Critical Reviews in Biotechnol*ogy 26 121–143.
- Bude Ugarte M, Guglielmotti D, Giraffa G, Reinheimer J and Hynes E (2006) Nonstarter lactobacilli isolated from soft and semihard Argentinean cheeses: genetic characterization and resistance to biological barriers. *Journal of Food Protection* 69 2983–2991.
- CAA (2006) Código alimentario Argentino [Food Code of Argentina] [Internet document] URL 'http://www.anmat.gov.ar/alimentos/normativas\_alimentos\_caa.asp. Accessed 30/11/2011.
- Candioti M C, Hynes E, Quiberoni A, Palma S B, Sabbag N and Zalazar C A (2002) Reggianito Argentino cheese: influence of *Lactobacillus helveticus* strains from natural whey cultures on cheese making and ripening processes. *International Dairy Journal* 12 923–931.
- Ceruti R J, Zorrilla S E and Sihufe G A (2012) The influence of elevated initial ripening temperature on the proteolysis in Reggianito cheese. *Food Research International* **48** 34–40.

- Coppola R, Nanni M, Iorizzo M, Sorrentino A, Sorrentino E, Chiavari C and Grazia L (2000) Microbiological characteristics of Parmigiano Reggiano cheese during the cheesemaking and the first months of the ripening. *Lait* 80 479–490.
- Drake M A and Delahunty C M (2011) Sensory evaluation. In *Encyclopedia of Dairy Sciences*, Vol. 1, pp 279–283. Fuquay J W, Fox P F and McSweeney P L H, eds. London: Academic Press.
- Ferrazza R E, Fresno J M, Ribeiro J I, Tornadijo M E and Mansur Furtado M (2004) Changes in the microbial flora of Zamorano cheese (P.D.O.) by accelerated ripening process. *Food Research International* **37** 149–155.
- Folkertsma B, Fox P F and McSweeney P L H (1996) Accelerated ripening of Cheddar cheese at elevated temperatures. *International Dairy Journal* 6 1117–1134.
- Giraffa G, Mucchetti G, Addeo F and Neviani E (1997) Evolution of lactic acid microflora during Grana cheese-making and ripening. *Microbiologie – Aliments – Nutrition* 15 115–122.
- Hannon J A, Wilkinson M G, Delahunty C M, Wallace J M, Morrisey P A and Beresford T P (2005) Application of descriptive sensory analysis and key chemical indices to assess the impact of elevated ripening temperatures on the acceleration of Cheddar cheese ripening. *International Dairy Journal* 15 263–273.
- Hough G, Martinez E, Barbieri T, Contarini A and Vega M J (1994) Sensory profiling during ripening of Reggianito grating cheese using both traditional ripening and in plastic wrapping. *Food Quality and Preference* 5 271–280.
- Hynes E R, Bergamini C V, Suárez V B and Zalazar C A (2003) Proteolysis on Reggianito Argentino cheeses manufactured with natural whey cultures and selected strains of *Lactobacillus helveticus*. *Journal of Dairy Science* 86 3831–3840.
- IDF (1969) Determination of the Fat Content of Cheese and of Processed Cheese Products. IDF standard 5A. Brussels, Belgium: IDF.
- Law B A (2001) Controlled and accelerated cheese ripening: the research base for new technologies. *International Dairy Journal* 11 383–398.
- McSweeney P L H (1997) The flavour of milk and dairy products: III. Cheese: taste. *International Journal of Dairy Technology* **50** 123–128.
- O'Mahony J A, Sheehan E M, Delahunty C M and McSweeney P L H (2006) Lipolysis and sensory characteristics of Cheddar cheeses ripened using different temperature-time treatments. *Lait* **86** 59–72.
- Perotti M C, Bernal S M, Meinardi C A and Zalazar C A (2005) Free fatty acid profiles of Reggianito Argentino cheese produced with different starters. *International Dairy Journal* **15** 1150–1155.
- Reinheimer J A, Quiberoni A, Tailliez P, Binetti A G and Suarez V B (1996) The lactic acid microflora of natural whey starters used in Argentina for hard cheese production. *International Dairy Journal* 6 869–879.
- Sihufe G A, Zorrilla S E and Rubiolo A C (2003) Casein degradation of Fynbo cheese salted with NaCl/KCl brine and ripened at various temperatures. *Journal of Food Science* 68 117–123.
- Sihufe G A, Zorrilla S E, Mercanti D J, Perotti M C, Zalazar C A and Rubiolo A C (2007) The influence of ripening temperature and sampling site on the lipolysis in Reggianito Argentino cheese. *Food Research International* **40** 1220–1226.

- Sihufe G A, Zorrilla S E and Rubiolo A C (2010a) The influence of ripening temperature and sampling site on the proteolysis in Reggianito Argentino cheese. *LWT – Food Science and Technology* **43** 247–253.
- Sihufe G A, Zorrilla S E, Sabbag N G, Costa S C and Rubiolo A C (2010b) The influence of ripening temperature on the sensory characteristics of Reggianito Argentino cheese. *Journal of Sensory Studies* 25 94–107.
- Sihufe G A, Zorrilla S E, Perotti M C, Wolf I V, Zalazar C A, Sabbag N G, Costa S C and Rubiolo A C (2010c) Acceleration of cheese

ripening at elevated temperature. An estimation of the optimal ripening time of a traditional Argentinean hard cheese. *Food Chemistry* **119** 101–107.

- Sihufe G A, Rubiolo A C and Zorrilla S E (2012) Reggianito cheese hard cheese produced in Argentina. In *Handbook of Animal-Based Fermented Food and Beverage Technology*, pp 377–386. Hui Y H, ed. Boca Raton, FL: CRC Press.
- Zorrilla S E and Rubiolo A C (1994) Fynbo cheese NaCl and KCl changes during ripening. *Journal of Food Science* **59** 972–975, 985.