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The influence of elevated initial ripening temperature on the proteolysis in Reggianito cheese

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ABSTRACT

The effects of elevated initial ripening temperature on proteolysis in Reggianito cheese were evaluated considering 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 analysis, urea-PAGE analysis of the urea-soluble fraction, RP-HPLC analysis on the water-soluble fraction at pH 4.6, and free amino acid analysis. In general, increasing ripening temperature and time resulted in increases of proteolysis products, notably higher levels being observed in experimental cheeses initially stored at 20 °C for 4 weeks. Principal component analysis showed that those cheeses at 124 days of ripening had similar levels of proteolysis products to the control cheeses at 180 days of ripening. In conclusion, promising results related to the proteolysis event in Reggianito cheese were obtained, which may help in the selection of a convenient elevated temperature–time combination for accelerating its ripening.

1. Introduction

Proteolysis is considered the most complex, and for many varieties of cheese, the most important event among all chemical and biochemical pathways occurring in cheese during ripening (Forde & Fitzgerald, 2000). The process is characterised by a sequential breakdown of the caseins into smaller fragments. In a primary proteolysis, caseins are broken into large and medium sized peptides, mainly as a consequence of the action of residual chymosin and plasmin. In a secondary proteolysis, a subsequent degradation of those peptides, mainly due to the action of proteases and peptidases coming from different microorganisms occurring in cheese, releases small peptides and free amino acids, which in turn can be degraded to catabolic products (McSweeney & Sousa, 2000).

Several attempts have been made in order to accelerate cheese ripening, since this is a long and thus expensive step of cheese manufacturing, particularly in low moisture, slow-ripening varieties (Folkertsma, Fox, & McSweeney, 1996). Increasing storage temperature is the simplest method available for the acceleration of cheese ripening period, and it has the additional benefit of potential savings resulting from lower refrigeration costs (O'Mahony, Sheehan, Delahunty, & McSweeney, 2006). Main drawbacks mentioned for this technology are an increased risk of development of undesirable microorganisms and non-specific increases in ripening reactions that can lead to the presence of unbalanced flavours or off-flavours, together with potential body defects such as softening or crumbliness (Law, 2001).

Reggianito cheese is the most important hard cheese variety mainly produced in the central and east-central regions of Argentina (known as the Pampas region), being extensively consumed locally and also exported to several countries worldwide. Its origin is an adaptation of cheesemaking technologies of hard Italian cheeses Grana Padano and Parmigiano Reggiano, brought to the country by Italian immigrants in the late 19th and early 20th centuries. It is manufactured with pasteurised cow milk and natural whey starter is used, 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 and 82–85% relative humidity. According to CAA (2006), Reggianito 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. In relation to ripening acceleration of Reggianito cheese, different studies have been carried out to assess the impact of an elevated storage temperature of 18 °C for 6 months on the lipolysis (Sihufe et al., 2007), proteolysis (Sihufe, Zorrilla, & Rubiolo, 2010) and sensory characteristics (Sihufe, Zorrilla, Sabbag, Costa, & Rubiolo, 2010), as well as a statistical analysis taking into account all that information (Sihufe, Zorrilla, Perotti, et al., 2010). In addition to characterise main transformations occurring during ripening, those studies allowed establishing an optimal ripening period between 2 and 3 months when cheeses are stored at 18 °C.

As claimed in previous works (Aston, Fedrick, Durward, & Dulley, 1983; Ferrazza, Fresno, Ribeiro, Tornadijo, & Mansur Furtado, 2004; Hannon et al., 2005; O'Mahony et al., 2006), application of high

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storage temperatures during the initial stages of ripening appears to cause a moderate and equilibrated increase in the activity of enzymatic systems, whereas a reduction in the risk of microbiologic contamination or unbalance in biochemical reactions could be achieved. Consequently, application of higher initial storage temperatures emerges as an interesting alternative instead of treatments based on ripening at high temperatures during the whole ripening period. In the present study, our objective was to evaluate the effect of different temperature–time combinations during ripening on the proteolysis in Reggianito cheese.

2. Materials and methods

2.1. Cheese sampling and ripening conditions

Twenty cheeses (7.8 ± 0.1 kg weight, 23.7 ± 0.2 cm diameter, 15.3 ± 0.2 cm height) manufactured with milk from the same cheese vat and by a standard cheese making procedure were brought from a local factory to our laboratory. Two cheeses were used to determine their initial composition, while the other 18 cheeses were stored at 3 different temperature–time combinations for 6 months. As listed in Table 1, 6 cheeses were ripened at 12 °C and 85% relative humidity, while the other 12 cheeses were ripened at two different temperature–time combinations, also at 85% relative humidity. Cheeses were analysed in duplicate at 61, 124 and 180 days of ripening.

2.2. Physicochemical analysis

Samples were completely grated and analysed to determine the moisture and chloride content (Zorrilla & Rubiolo, 1994), total nitrogen (TN), water-soluble nitrogen at pH 4.6 (WSN) (Sihufe, Zorrilla, & Rubiolo, 2003). For pH determination, a pH electrode for solid foods was used (pH Spear, Oakton Instruments, Vernon Hills, IL, USA). Fat content was determined for initial composition (International Dairy Federation, 1969). Maturation index (MI) was expressed as a percentage of WSN of the cheese TN (WSN × 100/TN). Determinations were carried out in duplicate, except for chloride content that was determined in triplicate.

2.3. Electrophoretic analysis

Electrophoretic analysis was performed as described by Sihufe, Zorrilla, and Rubiolo (2010). Cheese fractions were obtained dissolving grated cheese (3 g) in 25 mL of 8.66 mol/L urea, in a procedure including fat remotion by cold filtration and centrifugation. Electrophoretic runs of the fractions were made on vertical discontinuous polyacrylamide gels using anodic buffers. The current was set at a constant value of 50 mA and Coomasie blue R250 was used to stain the gels. Stained gels were scanned, and using Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD, USA) images were processed to obtain relatives areas for each band of interest. Standards of α_{s1}-casein and β-casein (Sigma Chemical Co., St Louis, MO, USA) were run in 2 lanes of each gel.

2.4. Peptide analysis

The extraction of the water-soluble fraction at pH 4.6 (WSF) was performed as suggested by Sihufe et al. (2003). The WSF (100 μL) filtered through a disposable 0.2-μm filter were injected in the chromatograph. Equipment and chromatographic conditions for peptide analysis by RP-HPLC were the same as described by Sihufe, Zorrilla, and Rubiolo (2010), but separation was performed on a Microsorb-MV (250 × 4.6 mm) C18, 300 Å column (Varian Inc., Palo Alto, CA, USA).

Table 1
Summary of the ripening conditions used.

Cheese code	Ripening conditions
C	12 °C for 6 months
E_1	20 °C for 2 weeks followed by 12 °C up to 6 months
E_2	20 °C for 4 weeks followed by 12 °C up to 6 months

2.5. Free amino acid analysis

Soluble fractions in 2.5 g/100 mL sulfosalicylic acid (SSA-SF) were obtained from the WSF. Free amino acids were determined in the SSA-SF using the derivatizing procedure with o-phtaldialdehyde (OPA) as described by Verdini, Zorrilla, and Rubiolo (2002). The resulting solution was filtered through a disposable 0.2-μm filter and 10 μL of the extract were injected. A Waters chromatography system (Waters Corporation, Milford, MA, USA) was used, which consisted of: Waters 1525 Binary HPLC Pump, Waters 717plus autosampler, FL-2 fluorescence detector (Isco, Inc., Lincoln, NE, USA) and Waters Breeze System software. A Microsorb-MV (250 × 4.6 mm) C18, 100 Å column (Varian Inc., Palo Alto, CA, USA) at 40 °C was used for chromatographic separations. Separations were carried out at a flow rate of 1.3 mL/min using solvent A: tetrahydrofuran:methanol: 0.05 mol/L sodium acetate pH 5.9 (1:19:80), and solvent B: methanol: 0.05 mol/L sodium acetate pH 5.9 (80:20) (Jones, Pääbo, & Stein, 1981). The gradient program was: initial composition 0% B, isocratic step at 0% B for 1 min, linear step to 14% B in 5 min, isocratic step at 14% B for 5 min, linear step to 50% B in 5 min, isocratic step at 50% B for 4 min, linear step to 75% B in 6 min, isocratic step at 75% B for 4 min, linear step to 100% B in 6 min, and isocratic step at 100% B for 4 min. Amino acids were identified according to their retention times by comparison with a standard solution chromatogram.

2.6. Statistical analysis

For statistical analysis, ripening conditions and ripening time were selected as main factors for ANOVA, performed using Statgraphics (Statgraphics Inc., Rockville, MD, USA). 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 applied to reduce the dimensionality of data obtained from chromatograms. Essentially, PCA provides the means to reduce the often large number of interdependent (correlated) variables (e.g. peak areas) represented in the original data set to a few independent (uncorrelated) variables or principal components that are linear combinations of the original variables and explain most of the variation in the original data set (Coker, Crawford, Johnston, Singh, & Creamer, 2005). This analysis was carried out using Minitab (Minitab Inc., State College, PA, USA). Moreover, PCA with the correlation matrix was used. In this form of PCA, data set is scaled before the analysis (each variable has a mean of zero and a standard deviation of one). In this way, the variables are given equal weighting but capturing the effect of all the variables rather the few variables with a comparatively large internal variance (Coker et al., 2005).

3. Results and discussion

3.1. Physicochemical characteristics

The initial composition of cheeses was 40.1 ± 0.2% (w/w) moisture, 20.8 ± 1.5% (w/w) fat, 33.1 ± 0.3% (w/w) protein, nondetectable chloride, and the pH was 5.24 ± 0.02. Table 2 shows the average values for pH, moisture content, chloride content and maturation index obtained during ripening of Reggianito cheese for the treatments studied. There have been only slight differences in pH values during ripening. Final pH values were similar to those previously referred by