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Ripening control of salt-reduced Manchego-type cheese obtained by brine vacuum-impregnation

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Abstract

Vacuum-impregnation salting was applied to Manchego-type cheese in order to obtain a salt-reduced cheese. Cheese ripening was evaluated in the medium and internal zones throughout a 90 day ripening period.The parameters analysed were: pH, moisture, salt concentration, water-soluble nitrogen (WSN), TCA-soluble nitrogen (TCASN), free amino acids (NH₂-N), casein profile by electrophoresis, peptide profile by HPLC, textural characteristics by an uniaxial compression test and sensory analysis by means of a triangle and a preference test. Salt penetration was stronger in vacuum impregnated cheeses than in conventionally salted cheeses. Globally, the ripening stage was the main factor affecting cheese evolution, followed by cheese zone. Salting treatment only affected peptide profile evolution and cheese texture. Vacuum-impregnated cheeses presented a higher hydrophilic and a lower hydrophobic peptide content and became less fracturable. \odot 2000 Published by Elsevier Science Ltd. All rights reserved.

1. Introduction

Cheese salting is one of the main steps in cheese manufacture. Salt fulfills many important functions in cheese: it contributes directly to cheese flavour, it controls the growth of starter and nonstarter bacteria, it regulates the activity of rennet and other enzymes, and it promotes curd syneresis (Morris, Guinee & Fox, 1985).

Manchego-type cheese is the main ewe's milk cheese consumed in Spain. It is traditionally salted by immersion in a concentrated brine (Marcos, 1987) for a time that mainly depends on cheese volume and salting temperature.

The most frequent estimation of the minimum adult requirement for salt is 0.5 g of NaCl per day, while the average total daily salt intake by most people in the developed countries is $10-12$ g NaCl per day (IFT, 1980). Some studies have pointed out the important correlation existing between salt consumption and health disorders such as hypertension (Dillon, 1987; Van der Maten, van Raaij, Visman, van der Heijden & Oosterban Hautvast, 1997) and osteoporosis (Goulding,

Gold & Campbell, 1993). Therefore, consumers demand sodium reduced alternatives to traditional products.

Different procedures have been tested in order to reduce the salt content of cheese; e.g. Delveke, Paelich and Martens (1982) shortened the salting time. However, the most studied process has been the partial replacement of NaCl by other salt components obtained from potassium (Katsiari, Voutsinas, Alichanidis & Roussis, 1997, 1998), phosphate (Green, 1986) and magnesium and calcium (Fitzgerald & Buckley, 1985).

In previous papers, a new salting procedure was applied to Manchego-type cheese in order to determine its impact on cheese physicochemical and textural parameters during ripening (Guamis, Trujillo, Ferragut, Chiralt, Andrés & Fito, 1997; Pavia, Guamis, Trujillo, Capellas & Ferragut, 1999). This new salting method is based on the fast mass transfer mechanism that takes place during solid-liquid vacuum operations (Fito, 1994; Fito & Pastor, 1994). In the first step of vacuum impregnation, the gas occluded inside cheese pores flows out due to the effect of vacuum pressure. In the second step, atmospheric pressure is applied again to the system and brine flows easily into the cheese, not only by capilarity but also by pressure gradient.

This new salting method allows a faster salting and makes the cheese present a more homogeneous initial salt profile, considerably increasing the amount of salt

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in the center of the cheese. Keller et al. (1974) suggested that some of the low salted cheese problems (such as bitterness) could be inhibited by developing a mechanism for infusing the center of the cheese with NaCl.

No research has ever been conducted on the manufacture of Manchego-type cheese with less than the normal salt content. The aim of this work is to study the feasibility of applying brine vacuum-impregnation to obtain salt-reduced Manchego-type cheese. Specifically, our aim is to compare vacuum-impregnated saltreduced Manchego-type cheeses and conventional saltreduced Manchego-type cheese, in order to determine if the stronger salt penetration observed in the former results in a different ripening pattern.

2. Materials and methods

2.1. Cheesemaking

Cheeses were processed at the Food Technology pilot plant at the Universitat Autonoma de Barcelona. Ewe's milk was supplied by a close dairy farm. Cheeses were manufactured under the same conditions as reported previously (Pavia et al., 1999). After pressing, cheeses (100 mm height, 180 mm diameter) were divided into two lots for salting with the two different procedures.

2.2. Experimental design

Two batches of cheese from different lots of milk were considered for this study. Three cheeses from each salting procedure were ripened. Two of these three cheeses were sampled at different stages of maturation $(1, 7, 15, 15)$ 30, 60, 90 days). A representative portion of these cheeses was cut into symmetrical concentric sectors as showed in a previous paper (Guamis, Trujillo, Ferragut, Chiralt, Andrés & Fito, 1997) to get three different zones: the rind, the medium and the internal zones. After cutting the sectors from each cheese, the open surface was covered with a paraffin film to avoid anomalous drying, and ripening proceeded. The remaining cheese was kept intact to be sampled on day 90 for textural and sensory analysis.

2.3. Salting treatments and ripening

Conventional salting was performed by immersion in brine $(24\%$ NaCl solution) for 60 min at 10 \degree C. The vacuum-impregnation of cheese was carried out with 24% (w/w) brine in an apparatus with temperature and pressure control, according to a patent procedure (Fito et al., 1993). After cheese immersion (at 10° C), a vacuum of 3.7 kPa (absolute) was applied for 30 min. The duration of the two salting procedures was established in preliminary trials so that the same global amount of salt was obtained in both types of cheeses. Cheeses were ripened in a chamber at 14° C and 80% relative humidity.

2.4. Cheese analysis

The pH was determined on a slurry obtained by homogenizing 10 g of cheese and 10 ml of deionized water in pH meter (Crison MicropH 200L; Crison Instrument, Barcelona, Spain). Sodium Chloride was determined with a Chloride Analyzer (926 Chloride Analyzer, Sherwood Scientific Ltd., Cambridge, UK). Moisture was determined according to the IDF standard (IDF, 1982). All analyses were performed in duplicate.

2.5. Nitrogen fractions

The cheese N was fractionated according to the method of Kuchroo and Fox (1982). The extracts thus obtained were used to determine the water-soluble N at pH 4.6 (WSN) and the soluble N in 12% trichloroacetic acid (TCASN) as a percentage of the acid in the final solution. Total N (TN), WSN and TCASN were determined by the Kjeldahl method (IDF, 1993) with Kjeltec equipment (1026 Distilling Unit, Tecator, Höganas, Sweden). The evolution of total free amino acids $(NH_{2}+$ N) was monitored according to Folkertsma and Fox (1992) using the Cd-ninhydrin reagent (Sigma-Aldrich Chemie, Stenheim, Germany). All analyses were performed in duplicate.

2.6. Electrophoresis analysis

Precipitates of protein obtained from N fractionation were washed twice in distilled water, centrifuged and freeze-dried. Five milligrams were dissolved in 1 ml of 7 M urea, and 25μ of 0.05% aqueous solution of bromophenol blue were added. Samples of 15 µl of this solution were taken for the electrophoretic separations. Alkaline urea-PAGE with 1 mm spacers was performed according to Akroyd (1968) with 8.8% T (g acrylamide + g bis-acrylamide $\times 100$ ml⁻¹), 2.3% C (g bisacrylamide \times % T⁻¹) and 5M urea at pH 8.9 as described by Carretero, Trujillo, Mor-Mur, Pla and Guamis (1994). Gels were stained with Coomassie Blue R-250 (Sigma Chemical Company, St Louis, MO) (Uriel, 1966) and were destained by repeated washing in ethanol, acetic acid, glycerol and water (200:50:25:725, v/v) solution.

2.7. Peptide analysis by HPLC

Peptides from the water-soluble extract were separated by RP-HPLC using an automated Waters system (LCM1, Waters Corporation, Milford, MA, USA). All separations were carried out on a 250×4.6 mm column packed with C18-bonded silica gel, wide-pore of 3000 nm and particle diameter of 5 μ m (Symmetry 300TM, Waters Chromatography, Milford, MA, USA) at a constant temperature of 40° C, following the method proposed by González del Llano, Polo and Ramos (1995). After running the samples, the integration area of peptides, excluding that of free amino acids, was determined and divided into groups to allow development of a quantitative hydrophobic and hydrophilic peptide content, following the same criteria as other authors (González del Llano et al., 1995). Results for the amounts of hydrophobic and hydrophilic peptides were expressed as units of chromatogram area per gram of dry cheese.

2.8. Texture analysis

Cube-shaped samples (1000 mm³) of intact 90-day old cheeses were cut and held at 7° C for 4 h prior to carrying out the uniaxial compression test. Cubes were compressed to 80% of their original height at a constant temperature of 7° C using a TA-TX2 Texture Analyzer (State Micro System, Survey, UK) with a 25 kg load cell and a crosshead speed of 50 mm min^{-1} . The analysis was carried out eight times for each cheese. All the experiments were conducted under lubricated conditions (Casiraghi, Bagley & Christianson, 1985).

2.9. Sensory analysis

Samples of Manchego-type cheese were cut into pieces of about $40 \times 20 \times 10$ mm in size and placed on white plates coded with three-digit random numbers. Water was provided for mouth washing between samples. The cheeses were evaluated organoleptically after 90 days of ripening by a 14 member panel familiar with Manchegotype cheese. A triangle test was developed to evaluate the capability of the panel to distinguish between cheeses salted by both procedures. Afterwards, a preference test was done to assess which cheese was preferred by the panel.

2.10. Statistical analysis

Cheese age, salting treatment and sample location were the main studied effects, except in textural analysis. where salting treatment was the only studied effect. Data from the two batches were analyzed using ANOVA of the General Linear Models procedure of SAS[®] software (SAS Institute, Inc., Cary, NC27513). Level of significance was set for $P < 0.05$. Results from sensory analysis were evaluated according to Stone and Sidel (1993).

3. Results and discussion

3.1. Cheese analysis

According to Marcos, Fernández-Salguero, Esteban, León, Alcalá and Beltrán Heredia (1983), the mean salt value of commercial Manchego-type cheese is 2.32%. In our study, the final salt value of Manchego-type cheese at the end of ripening was approximately one third of that (0.8%). As expected, salt penetration was stronger in VI (vacuum-impregnated) cheeses than in CS (conventionally-salted) cheeses (Table 1), although global salt-in-moisture (S/M) concentration was almost the same $(1.11$ and 1.08 for CS and VI cheeses, respectively). On the first day after manufacturing, S/M concentration in the medium and internal zones for VI cheeses were higher than for CS cheeses, whereas S/M concentration in VI rind was lower than in CS rind. This different salt profile remained on day 7. On day 15, only the internal zone of VI cheeses presented a higher S/M content. Salt equilibrium was reached on day 30. From then on, no differences in S/M were observed due to cheese zone or salting method.

Some authors have pointed out the significance of $S/$ M in lactic acid production and pH (Fox, Lucey & Cogan, 1990; Thomas & Pearce, 1981). In our study, pH was significantly affected $(P < 0.05)$ by ripening stage (Table 1). Values of pH decreased from the first day to day 15, increasing slowly from then on. Neither cheese zone nor salting treatment affected pH evolution.

Moisture decreased from day 1 to day 90 (Table 1). Cheese age was the main significant factor affecting moisture content evolution, followed by cheese zone. The internal zone presented a higher moisture content than the medium one from day 30 to day 90. This higher moisture content of the internal zone can be explained by the continuous water evaporation from the cheese surface that takes place during ripening (Van der Berg & Exterkate, 1993).

3.2. Nitrogen fractions

Rennet, and to lesser extent plasmin, are the main proteolytic agents responsible for the hydrolysis of caseins to large and intermedium size peptides (Desmazeaud & Gripon, 1977; Furtado & Partidge, 1988). Most of the soluble nitrogenous components found in WSN are produced by the action of rennet, though other cheese components are also soluble in this fraction, such as proteose peptone and whey proteins (Rank, Grappin & Olson, 1985).

The ripening stage was the main significant factor affecting WSN evolution. WSN regularly increased from day 1 to day 90 (Table 1). The evolution of WSN was also affected by cheese zone. The medium zone

presented a significantly higher WSN content than the internal one on day 60 and day 90. This could be related to the lower moisture content (and the higher dry matter content) of the medium zone at the end of ripening. Guinee and Wilkinson (1992) asserted that changes in the rennet-to-casein ratio give rise to variations in the residual rennet activity. In our cheese, the higher water evaporation of the medium zone with respect to the internal one could act to increase the residual rennet-tocasein ratio, which in turns would result in a higher WSN content. When comparing this salt-reduced Manchego-type cheese with other Manchego-type cheese with the traditional salt content (Guamis et al., 1997; Pavia et al., 1999), the latter presented a slightly higher WSN concentration. A possible explanation of this could be the lower α_{s1} -casein hydrolysis that saltreduced Manchego-type cheese presents in relation to traditionally salted Manchego-type cheese.

Starter bacteria are primarily responsible for the small size peptides, amino acids, ammonia and other minor compounds that are soluble in 12% TCA (Desmazeaud & Gripon, 1977; Furtado & Partidge, 1988). Cheese age was the main significant factor affecting TCASN evolution, followed by cheese zone. The salting procedure did not affect TCASN concentration during ripening, which increased from the first day to day 90 (Table 1). At the end of the studied ripening period, the medium zone presented a higher TCASN content than the internal one due to the higher WSN content of this zone. When expressing the TCASN in relation to the WSN, no differences were observed because of cheese zone. The maximum TCASN value (approximately 80% of WSN) was attained by day 90 and was higher than that obtained in Manchego-type cheese with the traditional salt content (Guamis et al., 1997), probably due to the higher starter activity observed in cheeses with low S/M values.

 $NH₂$ $-N$ concentration was estimated by means of the Cd-ninhydrin method, which is highly sensitive to free amino acids. Ripening stage was the only factor affecting amino acid evolution. Amino acid concentrations increased from day 1 to day 90 (Table 2). However, no significant differences were observed from day 1 to day 15. The medium zone presented a slightly higher amino acid concentration than the internal one (derived from the higher WSN content of the first), but differences were not statistically significant.

3.3. Electrophoretic analysis

Primary proteolysis in cheese may be defined as those changes in β -, γ -, α_s -caseins, peptides an other minor bands, which can be detected by PAGE (Rank et al., 1985). Fig. 1 shows the electrophoretograms for the casein fraction of the medium zone of a CS cheese on different stages of ripening. No major qualitative differences between cheeses salted by the two treatments were observed. The other electrophoretograms were obviated because they presented similar results to this one. The hydrolysis of α_s -casein was more extensive than the hydrolysis of b-casein, as corresponds to bacterialripened cheeses. The α_{s1} -casein was initially hydrolyzed to α_{s1} -I-peptides, which were observed from the first day after manufacturing to the end of the studied ripening period. When comparing these electrophoretograms with those obtained from Manchego-type cheeses with traditional salt contents (Guamis et al., 1997), a higher α_{s1} -casein hydrolysis was observed in the latter. Some authors (Fox & Walley, 1971) observed that α_{s1} -casein was optimally degraded by chymosin in the presence of 5% S/M. These values were not achieved during ripening of salt-reduced Manchego-type cheeses, whereas they were obtained in traditional Manchego-type cheeses 60 days after manufacturing.

Day 90 Medium 20.3 (0.17) 20.1 (0.46) 16.7 (1.21) 16.6 (0.63) 3.15 (0.23) 3.30 (0.11)

Internal 16.7 (0.64) 16.9 (0.16) 13.4 (0.39) 13.4 (0.09) 1.59 (0.20) 1.55 (0.15)

Internal 17.9 (0.15) 18.4 (0.01) 15.4 (0.12) 15.5 (0.07) 2.41 (0.12) 2.70 (0.01)

Mean (and standard deviation) values of nitrogen fractions and free amino acids of experimental conventional-salted (CS) and vacuum-impregnated

Table 2

Fig. 1. Urea-PAGE electrophoretograms of the medium zone of conventional salted cheeses at 1, 7, 15, 30, 60 and 90 days of ripening.

Some minor bands with an electrophoretic mobility greater than β -casein but lower than α -casein were observed. The intensities of these bands increased during ripening. These bands were not observed in the electrophoretograms of traditionally-salted Manchegotype cheese (Guamis et al., 1997) and could correspond to β -casein degradation products. Hydrolysis of β casein by rennet is strongly inhibited by S/M (Fox $\&$ Walley, 1971). This could explain the presence of these bands in the salt-reduced cheeses and their absence in the traditionally-salted Manchego-type cheese.

3.4. Peptide analysis by HPLC

HPLC, using reversed phase, has been studied as a method for separation of peptides in cheese extracts (Ardö $& Gripon, 1991$). In the present work, a gradient elution solvent was used to separate peptides and amino acids, depending on their hydrophobicity. This means that amino acids and hydrophilic components eluted first and that more hydrophobic components eluted later. Both hydrophobic and hydrophilic peptides were significantly affected by ripening stage, cheese zone and salting treatment. Globally, hydrophobic peptide content increased from the first day to day 60 (except from day 15) to day 30, where no significant differences were observed), decreasing afterwards (Table 3). Hydrophilic peptide concentration increased throughout the studied ripening period. The medium zone of cheeses salted by both treatments presented a higher hydrophilic and a lower hydrophobic peptide concentration than the internal one. On the other hand, CS cheeses showed lower hydrophilic and higher hydrophobic peptide contents than VI cheeses.

When expressing the amount of hydrophobic peptides in relation to the amount of hydrophilic peptides, the ratio decreased with increasing cheese age (Table 3). However, no significant differences were observed from day 7 to day 30 or from day 60 to day 90. Lau, Barbano and Rasmussen (1991), González del Llano et al. (1995) and Mohedano, Fernández, Gaya, Medina and Núñez (1998) also observed that hydrophobic to hydrophilic peptide ratio decreased during ripening in Cheddar cheese, Afuega'l Pitu cheese and Hispanico cheese, respectively. Cheese zone and salting treatment also affected peptide ratio evolution. The medium zone presented a lower peptide ratio than the internal one, whereas CS cheeses presented a higher peptide ratio than VI cheeses. Lawrence and Gilles (1969) and Stadhouders, Hup, Exterkate and Visser (1983) asserted that S/M is one of the most important factors that determine bitterness in cheese: cheeses with low salt concentration are acid and bitter. Several reports (Champion & Stanley, 1982) have indicated that peptides with high contents of hydrophobic side chains will develop a bitter taste. Some authors have observed that bitter peptides were mainly derived from β -casein hydrolysis (which is inhibited at high salt concentrations) (Fox & Walley, 1971; Phelan, Guinee & Fox, 1973). In our study, the relatively high initial S/M concentration of VI cheeses in Table 3 Mean (and standard deviation) values of water soluble peptides of experimental conventional salted (CS) and vacuum impregnated (VI) cheeses during ripening

relation to CS cheeses could act to limit hydrophobic peptide production or enhance the degradation of hydrophobic to hydrophilic peptides. However, an indepth study is needed to explain the mechanism of this different peptide profile.

3.5. Texture analysis

True stress and true strain values were calculated according to Hort, Grys and Woodmen (1997). Two parameters were calculated from true stress-true strain curves: fracture stress (σ_f), and fracture strain (ε_f). Fracture stress, which indicates low fracturability (Fredrick & Dulley, 1984; Kfoury, Mpagana & Hardy 1989), was significantly higher in VI cheeses than in CS cheeses (Fig. 2). Pavia et al. (1999) observed that the VI process acted to compact the protein matrix and this could result in a lower cheese fracturability. Fracture strain, which is an estimation of cheese shortness (Kfoury et al., 1989) was higher in VI cheeses than in CS cheeses. This difference, however, was not statistically significant. Texture values observed in salt-reduced Manchego-type cheese were lower than those observed in traditionally-salted Manchego-type cheese (Pavia et al., 1999), indicating the more fracturable and weak texture of this salt-reduced cheese. However, VI salt-reduced cheeses presented a texture more similar to traditionally-salted Manchegotype cheeses than CS salt-reduced cheeses.

3.6. Sensory analysis

The panel could accurately distinguish between cheeses salted by the two treatments ($P \le 0.001$). When asking for their preferences, VI cheeses were preponderantly chosen ($P < 0.15$). Voluntary comments indicated that VI cheeses presented a firmer texture and a lower bitterness than CS cheeses.

Fig. 2. Stress-strain figure of conventional brine-salted cheeses (thin line) and vacuum-impregnated cheeses (bold line).

4. Conclusions

Vacuum-impregnation allows a stronger penetration of salt into the centre of the cheese, and partially solves two of the most important problems of low-salted cheeses, which are the amounts of hydrophobic peptides and the loss of cheese structure. VI cheeses were less fracturable and contained less hydrophobic peptides than CS cheeses. The trained panel could distinguish between cheeses salted by the two treatments and preferred VI cheeses. All things considered, VI can be taken as an alternative way to obtain salt-reduced cheeses.

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