Proteolysis of Fynbo Cheese Salted with NaCl/KCl and Ripened at Two Temperatures

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ABSTRACT

Cheeses salted in solutions of 100g NaCl/L and 100g KCl/L and ripened for 90 days at 12°C and 16°C were compared with cheeses salted in brine of 190g NaCl/L and ripened at the same temperatures. Peptides of the water-soluble nitrogen (WSN) fraction were quantified by the Kjeldahl method and analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) to follow the course of proteolysis. There were no differences (P>0.05) attributable to salt treatments; and the cheeses stored at 16°C showed higher levels of WSN/Total Nitrogen (TN) than cheeses ripened at 12°C. HPLC profiles of WSN extracts showed differences in the hydrophilic zone for cheeses ripened at different temperatures, but profiles were similar for cheeses salted with NaCl and with NaCl/KCl.

Key Words: cheese, proteolysis, potassium chloride, ripening temperature, HPLC

INTRODUCTION

DURING CHEESE RIPENING, A PART OF THE casein is converted by proteolysis into watersoluble nitrogenous compounds, such as peptides and amino acids, which contribute to the flavor and texture (Fox, 1989). The proteolysis depends on the salt-in-moisture level. NaCl influences cheese ripening mainly through its effect on water activity; however, it also affects bacterial growth, enzyme activities, synergism and physical changes in proteins which influence texture, protein solubility and probably, protein conformation (Guinee and Fox, 1987). In cheeses salted by brine immersion, NaCl diffuses from the surface to the center until it reaches uniformity of distribution.

High salt intake may be of concern to people with abnormal blood pressure, since in such cases an excessive ingestion of sodium is not advisable. Furthermore, controlled studies have shown that an increase of sodium in the human diet is accompanied with higher excretion of calcium in the urine (Weaver and Evans, 1986). Some evidence has suggested that a lower Na:K ratio may be more important than the absolute level of Na in the regulation of hypertension (Weaver and Evans, 1986). Therefore, a common approach has been to replace NaCl by KCl because of the similarity of physical and functional properties, although the substitution is usually partial because KCl is more bitter than NaCl.

Reddy and Marth (1993) studied the proteolysis in Cheddar cheese salted with NaCl, KCl and their mixtures and concluded that the use of KCl did not result in bitterness. Nevertheless, whenever the starter bacteria or other factors cause formation of bitter peptides, the KCl might not inhibit or mask development of bitterness to the same extent as NaCl. Iwanzczak et al. (1995) analyzed the possibility of increasing the potassium content in ripened cheeses and successfully applied a mixture of NaCl and KCl (1:1) for salting some varieties of cheeses.

Studies of Fynbo cheese salted with KCl/NaCl have been reported by Zorrilla and Rubiolo (1994a, b) who modeled the KCl and NaCl movement during salting and diffusion during ripening. They also studied proteolysis analyzing the degradation of major caseins by electrophoresis and by HPLC (Zorrilla and Rubiolo, 1997; Zorrilla et al., 1996). However, chromatographic studies of the water-soluble fraction of Fynbo cheese salted with KCl/NaCl mixtures have not been reported.

Temperature is an important factor that modifies biological and physical processes during ripening. Higher storage temperature shortens the period of ripening and reduces costs. Aston et al. (1983) and Fedrick et al. (1983) analyzed the influence of higher temperature in the initial and middle stages of ripening, respectively. Those conditions resulted in accelerated ripening. Fynbo cheeses are ripened for 30 days at 12°C before being readied for sale. The changes in proteolysis at higher temperatures have not been reported for sodium chloride or for potassium chloride.

The soluble nitrogen compounds are related to the development of typical texture, taste and flavor intensity (Aston and Creamer, 1986) and the soluble fraction of all extracts has been analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) to follow the course of proteolysis. Our objective was to determine and quantify soluble peptides as related to differences in salting with NaCl and with a mixture of NaCl/KCl (1:1) and also any effects of maturation temperature.

MATERIAL & METHODS

MINI FYNBO LOW-FAT CHEESES WERE PRODUCED by enzymatic (rennet) coagulation with a starter mixture of Lactobacillus helveticus, L. delbruekii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus. They were cylindrical semi-hard cheeses weighing 880-890g, 12 cm in diameter and 6.5 cm in height. The initial proximate composition before salting, determined using procedures cited by Zorrilla and Rubiolo (1991), was: moisture, 53.5%; protein, 33.0%; fat, 11.5% and NaCl, 0.42%.

Commercial Fynbo cheese samples were transported from the factory (SanCor Unit ed Cooperatives Ltd. in Gálvez, Santa Fe, Argentina) in a plastic vacuum bag and salted in our laboratory. Twelve cheeses were salted in a brine of 190g NaCl/L and 0.55% Ca++ at pH 5 (cheeses S) while twelve other cheeses were salted in a solution of 100g NaCl/L, 100g KCl/L and 0.55% Ca++ at pH 5 (cheeses K) salting them for 10h at 12°C and providing agitation with an air diffuser. When the cheeses were taken out of the salting solution they were packed under vacuum in heat-shrinkable plastic bags.

During ripening, six cheeses S and six cheeses K were stored at 12°C and the remainder at 16°C. Cheeses S and K stored at 12°C and at 16°C were sampled at 1, 10, 20, 30, 60 and 90 days of ripening.

PH 4.6 WATER-SOLUBLE NITROGEN FRACTION

The water-soluble fraction was prepared by grating 20g cheese and adding 70 mL water and homogenizing with a blender SB30 (Black & Decker, Australasia Pty. Ltd.) for 5 min. The mixture was held in a waterbath at 40°C for 1h. It was diluted to 100 mL and adjusted at pH 4.6 with HCl (0.5N). The homogenate was centrifuged (Biofuge 28RS, Heraeus Sepatech, Germany) for 10 min at 10,000×g and filtered through Whatman No. 42 paper.

Total nitrogen (TN) and water-soluble nitrogen (WSN) were determined using the micro-Kjeldahl method with an automatic digester model 430 and distillation unit model 322 (Büchi, Flawil, Switzerland) and a DL40RC titrator (Mettler Instrumente AG,
Fynbo Cheese Salted with NaCl/KCl ... Greifensee, Switzerland). Proteolysis, as an index of maturation (IM), was expressed as a percentage of WSN of the cheese TN (WSN/TN).

**Peptide analysis by HPLC**

A chromatograph (Isco, Inc., Lincoln, NE) with a gradient programmer model 2360, a V 4® variable wavelength absorbance detector and a SynChropak RPP (250’4.6 mm) C18, 300 Å column (SynChrom, Inc., Lafayette, IN) at 40°C were used. The method reported by González de Llano et al. (1995) was followed: Solvent A was 0.1% trifluoroacetic acid in water and solvent B was 0.1% trifluoroacetic acid in acetonitrile and water (60:40 vol/vol). Separations were carried out using 1 mL/min with solvent A for 10 min, a linear gradient from 0% to 80% of solvent B over 80 min and a mixture of solvents A and B (20:80) for 10 min. Detection was at 220 nm. Data were processed with the Chem Research Data System Program version 3.0.2. 1994 (Isco, Inc., Lincoln, NE).

All determinations were made in duplicate and ANOVA was applied to determine overall statistical differences of index of maturation by type of salting and temperature of ripening.

**RESULTS & DISCUSSION**

The indexes of maturation (IM) for different ripening times of cheeses S and K at 12°C and 16°C were compared (Fig. 1). Both cheeses showed increasing proteolysis with time during ripening. Other studies have clearly demonstrated that the increase of soluble peptides is due to the action of rennet at the initial ripening period in which αs1-casein is formed and further degraded by rennet and other proteinases during the first month of ripening (Law, 1987). However, the starter proteinases are more important to the secondary phase of proteolysis releasing small soluble peptides and free amino acids (Grappin et al., 1985).

The indexes of maturation, given by the ratio WSN/TN, of cheeses salted with NaCl/KCl mixtures did not differ (P > 0.05) from those of control cheeses S at any day or at the two temperatures. This confirmed results from physical analysis presented by Iwanczak et al. (1995) and Fitzgerald and Buckley (1985) which demonstrated that the combination of KCl with NaCl was the best alternative to reduce NaCl content in cheese.

The increase of WSN was faster during the first 30 ripening days of cheeses S and K reaching 66% of the 90 day value at 12°C and about 60% at 16°C. These results could probably be attributed to the breakdown of caseins, mainly αs1-casein, developed in the early stages of ripening, with a higher reaction rate than the others (Altemueller and Rosenberg, 1996).

When the indexes of maturation at 12°C and at 16°C were compared it was clear that the rate of production of soluble peptides increased as the temperature increased. The dif-
ference was more evident after 30 days of ripening. The degree of proteolysis in cheeses S and K ripened at 16°C for 20 days was similar to that in cheeses ripened at 12°C for 30 days (Fig. 1). The results confirmed previous assays (Aston et al., 1985) and showed that a higher storage temperature accelerated the rate of proteolytic breakdown, thus shortening ripening time.

RP-HPLC peptide profiles of the WSN of cheeses S and K which were ripened at 12°C and at 16°C were also compared (Fig. 2a and b). The chromatograms for cheeses S and K were similar for all days of ripening, with lower numbers and smaller peak areas during early stages of ripening but increasing quickly thereafter. These results indicated the presence and accumulation of new proteolysis products during ripening. The peaks that showed the most increases were eluted in the zones of 25, 30–40, 45, 62–68 and 72–80 min (horizontal lines, Fig. 2a and b). According to González de Llano et al. (1995) we assumed that the hydrophilic peptides were eluted from 10 to 35 min and hydrophobic peptides were those with retention times from 35 to 80 min. During ripening the relation between the hydrophilic and the hydrophobic zones was almost constant and close to 2.

When chromatograms were compared at different temperatures (Fig. 2a and b), the profiles were similar for 12°C and 16°C and only a marked difference was observed between 10 and 25 min. At 16°C these peaks appeared wider, more displaced at lower retention times and had poorer resolution. This suggested that at greater temperatures the proteolytic activity of the enzymes changed and different hydrophilic peptides were formed. The total areas of chromatograms of cheeses S and K at 12°C and at 16°C were compared (Fig. 3). Both the chromatographic method and the Kjeldahl method, showed the amount of soluble nitrogen products of cheeses ripened at 16°C for 20 days was the same as for cheeses ripened at 12°C for 30 days.

There were differences, at the two temperatures, between the curves of index of maturation (Fig. 1) and the curves of total area as a function of time (Fig. 3). Representing together the IM and the total chromatographic area in appropriate units during all studied periods, it is observed that there were almost the same slopes for both curves at 12°C for cheeses S and K (Fig. 4). At 12°C there existed a direct relationship between the quantification of soluble nitrogen by Kjeldahl and chromatographic methods. At 16°C a correspondence between the index of maturation of and the total area of chromatograms was also observed from day 1 to day 30, but after that time the increase of soluble compounds shown by chromatography was lower than those shown by the Kjeldahl method. In the first case the total soluble nitrogen was detected at 220 nm and the index of maturation was determined by the area of the peaks. The compounds formed at 16°C may have had less absorbency at 220 nm being more pronounced at 90 days of ripening. The hydrolysis of proteins may have been more extensive and the peptides more degraded to amino acids and other compounds that could not have been detected by that method.

CONCLUSIONS

SALT IS NECESSARY IN CHEESE RIPENING because of its influence on proteolytic activity of enzymes. The chromatographic method showed that partial substitution of Na by K did not affect the specific activity of proteases and peptidases. Pattern profiles of cheeses salted with NaCl and those salted with NaCl/KCl were very
similar in the number of peaks, retention times and areas. However, the Kjeldahl method showed that an increase in the temperature of ripening accelerated the proteolysis rate. The chromatographic method showed that the higher temperature modified the activity of proteases mainly in the formation of hydrophilic peptides.

REFERENCES
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