Casein Degradation of Fynbo Cheese Salted with NaCl/KCl Brine and Ripened at Various Temperatures

G.A. SIHUFE, S.E. ZORRILLA, AND A.C. RUBIOLO

ABSTRACT: Effect of temperature and salt substitution on casein degradation of Fynbo cheese was studied. Fynbo cheeses, salted in solutions of 190 g NaCl/L and of 100 g NaCl/L and 100 g KCl/L and ripened at 5, 12, and 16 °C, were sampled at 1, 5, 10, 20, 30, 60, and 90 d of ripening, at central and external zones. Samples were analyzed for moisture and chloride contents, maturation index, and casein degradation by urea-polyacrylamide gel electrophoresis. NaCl replacement by KCl did not affect any of the parameters studied. Total salt concentration and ripening temperature affected proteolysis significantly. First-order kinetics constants for α_{s1}-casein degradation were in the range of 0.002 to 0.016 day^{-1} and the activation energy of the reaction was approximately 26 kcal/gmol.

Keywords: Fynbo cheese, NaCl/KCl, ripening temperature, casein degradation, kinetics parameters

Introduction

FLAVOR, TEXTURE, AND APPEARANCE OF MOST OF THE CHEESE varieties are strongly influenced by the biochemical changes that occur during ripening such as proteolysis, lipolysis, and glycolysis. Proteolysis in particular is an essential change produced by proteinases of milk, and from added coagulants, lactic starter cultures, and other microorganisms. The activity of those agents varies due to the enzymes and medium conditions such as salt content and temperature.

Sodium chloride (NaCl), besides contributing to salty taste, has an important role in controlling cheese ripening, mainly through its effect on water activity. It also affects bacterial growth, enzyme activities, and syneresis (Guinee and Fox 1987). On the other hand, excessive dietary sodium is believed to contribute to hypertension and development of cardiovascular diseases. As a result, the food industry has responded to human dietary needs by providing processed foods, such as cheeses, with reduced amounts of sodium (Reddy and Marth 1991). Katsiari and others (2000) pointed out that when NaCl concentration in cheese is simply reduced, several drawbacks may occur such as an increase of proteolysis, water activity, acidity, and bitterness, as well as a decrease of firmness and saltiness or abnormal fermentations. Thus, for the production of low-Na cheese, the partial replacement of NaCl by KCl has been the most widely and successfully used alternative (Reddy and Marth 1993; Aly 1995; Katsiari and others 1998). On the other hand, a large salt gradient occurs in cheeses salted by brine immersion. As a result, salt diffuses from the surface to the center during ripening to eventually reach a uniform distribution. Salt movement is a slow process, and significant differences in concentration values can be observed at different cheese positions during ripening (Zorrilla and Rubiolo 1994). Therefore, it is also interesting to study proteolysis at cheese zones with different salt concentration history, such as central and external zones.

Temperature is also an important factor modifying biological and physical processes during ripening. Technically, storage of cheeses at elevated temperatures is the simplest method for accelerating ripening, but the risk of microbial spoilage and nonspecific reactions may increase and possibly lead to unbalanced flavor or off-flavors (Folkertsma and others 1996). Innocente and Corradini (1996) evaluated the effect of storage at 5 °C during the early stages of ripening on proteolysis and lipolysis of Montasio cheese to prevent the defect of swelling caused by clostridia bacteria. These authors have reported that the biochemical phenomena of ripening were retarded at low temperatures, but the typical organoleptic characteristics were not significantly altered when the low-temperature ripening period was shorter than 30 d. Folkertsma and others (1996) studied the effect of ripening at different time/temperature combinations on proteolysis, lipolysis, and flavor development of Cheddar cheese and concluded that ripening at 12 °C is more prudent, since the texture of cheeses ripened at 16 °C had deteriorated after about 6 mo. Although NaCl content and ripening temperature are important in cheese proteolysis, very little information about both factors is available.

Fynbo is a semihard cheese of either regular or low fat content, commonly salted for 10 h at 12 °C in a 20% NaCl brine solution and ripened for 30 d. Sodium chloride was partially replaced by KCl in Fynbo cheese and the effect of the replacement during ripening was studied (Zorrilla and Rubiolo 1997, 1999; Laborda and Rubiolo 1999). Comparisons were carried out considering a cheese salted with NaCl and a cheese salted with a mixture of NaCl/KCl. Typical values of saltiness and firmness were observed during ripening for both salting procedures. Although slight bitterness was detected in samples salted with NaCl/KCl brine, cheeses were considered acceptable for commercialization (Zorrilla and Rubiolo 1999). Zorrilla and Rubiolo (1997) did not observe differences in the kinetics of α_{s1}-casein degradation of cheeses salted in a NaCl or NaCl/KCl brine and ripened at 12 °C during the 1st mo of ripening. Chromatographic profiles of water-soluble nitrogen fractions were also very similar (Laborda and Rubiolo 1999). Moreover, it was reported that Fynbo cheeses stored at 16 °C showed higher values of water-soluble nitrogen/total nitrogen than cheeses ripened at 12 °C. Consequently, a thorough study about the effects of ripening temper-

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ature and salting procedure on cheese proteolysis will help to give a better understanding of ripening and to control the appropriate variables during cheese elaboration. Furthermore, kinetics studies should include the determination of kinetics parameters and its temperature dependence to improve temperature control of ripening or shelf life and to provide useful information for simulation or optimization processes. However, this type of data is hardly available. Our objectives were to study the casein degradation of Fynbo cheese at different ripening temperatures to determine characteristic kinetics parameters and to evaluate simultaneously the effect of partial NaCl replacement by KCl considering different cheese locations.

Materials and Methods

UNSALTED LOW-FAT FYNBO CHEESES (782.9 ± 27.4 G WEIGHT, 11.5 ± 0.3 cm dia, 6.1 ± 0.2 cm height) were brought from a local factory to our laboratory. Their initial composition was: 49.34 ± 0.29% (w/w) moisture, 29.72 ± 1.75% (w/w) protein, 12.56 ± 0.15% (w/w) fat, pH 5.15 to 5.35. Zorrilla (1993) and Zorrilla and Rubiolo (1997) did not observe significant differences in data obtained for different cheese vats. Therefore, 1 cheese vat was used in this study to reduce the number of cheeses assayed. Twenty-one cheeses were salted for 10 h at 12 °C in a solution of 190 g NaCl/L (cheese S) and 21 cheeses in a solution of 100 g NaCl/L and 100 g KCl/L (cheese K). Both brines also contained 0.55% Ca^{++} to prevent softening of cheese rind (Geurts and others 1972). After brining, each cheese was wiped and packed under vacuum in a heat-shrinkable plastic bag. During ripening, batches of 7 cheeses S and 7 cheeses K were stored at 5, 12, and 16 °C. Different cheeses were sampled at 1, 5, 10, 20, 30, 60, and 90 d. Slices (1 cm thickness) were cut parallel to the flat surface from the surface. Concentric rings of 9.9 cm minor dia and 12 cm major dia were cut from those slices (external zone). Slices (1.5 cm thickness) were cut parallel to the flat surface from the center. Cylindrical cores of 4.8 cm dia were cut from those slices (central zone). Samples obtained were completely grated and analyzed for moisture and chloride contents (Zorrilla and Rubiolo 1994), total nitrogen, water-soluble nitrogen, and casein degradation. All determinations were carried out in duplicate.

Water-soluble fraction extraction was performed with a modified procedure developed by Kuchroo and Fox (1982). Grated cheese (20 g) mixed with 30 mL water was homogenized using an ULTRA-TURRAX® T25 (IKA® Werke, Janke & Kunkel GmbH & Co KG, Staufen, Germany) homogenizer for 2 min. The homogenate was held at 40 °C for 1 h, pH was adjusted to 4.4 to 4.6, and the suspension was centrifuged at 4800 rpm and 5 °C for 30 min (Biofuge 28RS; Heraeus Sepatech, Osterode, Germany). After centrifugation, the upper layer of fat was removed. The supernatant, after being filtered through Whatman nr 42 paper (Maidstone, England) was diluted to 100 mL, constituting the water-soluble fraction. Total nitrogen (TN) and water-soluble nitrogen (WSN) were determined using the micro-Kjeldahl method with an automatic digestor model 430 and distillation unit model 322 (Büchi, Flawil, Switzerland) and a DL40RC titrator (Mettler Instrument AG, Greifensee, Switzerland). Index of maturation (IM) was expressed as a percentage of WSN of the cheese TN (WSN × 100/TN), and it was used to follow the proteolysis degree during ripening.

Electrophoretic analysis

Grated cheese (3 g) was dissolved in 25 mL of 8.66 M urea. Fat was removed by cold filtration and centrifugation before electrophoretic analysis. The zone electrophoretic method for whole casein or γ-casein fractions cited by McKenzie (1971) was used to obtain the polyacrylamide gel (7.5% acrylamide). Vertical anodic discontinuous buffers were used in LKB-2001 electrophoresis equipment (LKB Produkter AB, Bromma, Sweden). The constant current was set at 50 mA and Coomassie blue R 250 was used to stain the gels. Quantitative analysis of electrophoretic separations was performed by scanning the photograph at 632.8 nm using an LKB-2202 Ultroscan laser densitometer. A relative area was determined for each peak after scanning 3 tracks at different positions within the same sample band. The relationship between area and concentration was considered linear (Lesage and others 1993). Quantification of relative band densities was performed because the information related to all casein fractions selected can be evaluated simultaneously. Standards of α_{s1}-casein and β-casein (Sigma Chemical Co., St Louis, Mo., U.S.A.) were run in 2 lanes of each gel.

Statistical analysis

Data were analyzed using ANOVA. When differences between treatment effects were significant ($p < 0.05$), a multiple comparison of means was performed using the Tukey's test.

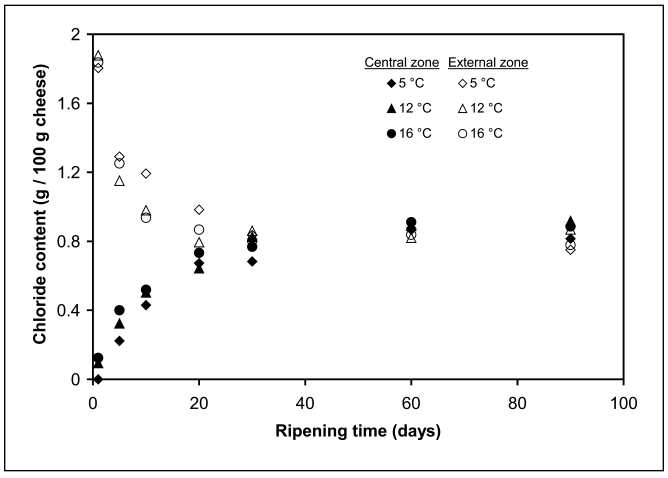

Figure 1—Profiles of chloride content during ripening of Fynbo cheeses salted with NaCl

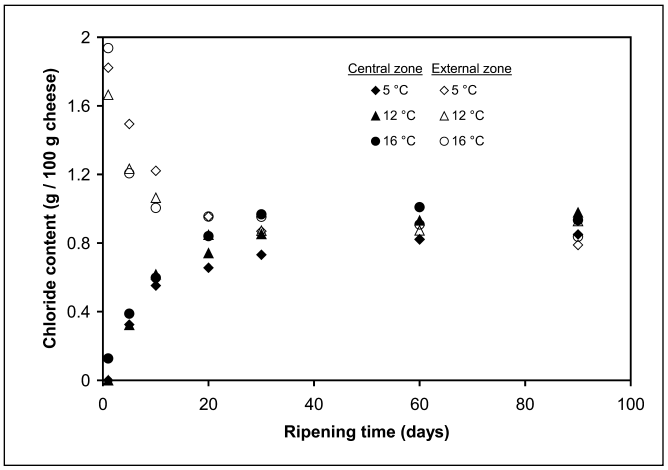

Figure 2—Profiles of chloride content during ripening of Fynbo cheeses salted with the mixture of NaCl/KCl

Results and Discussion

MEAN VALUES OF MOISTURE, CHLORIDE, AND MATURATION INDEX for Fynbo cheeses salted with NaCl or mixture of NaCl/KCl, ripened at different temperatures and sampled at various ages and zones, are shown in Figures 1 to 6.

Chloride and moisture contents

When cheeses are salted by brine immersion, salt is taken up and moisture is lost simultaneously. Moreover, taking into account that salt uptake and moisture loss are relatively slow processes, different concentrations at center and outside portions of the cheese at the end of the salting stage can be observed. These phenomena are clearly observed in both cheeses S and K (Figure 1 through 4). Concentration differences between central and external zones are notorious at 1 d of ripening. Those concentration gradients are the driving forces for the diffusion processes that occur during the ripening stage. In this case, cheeses were immediately covered with an impermeable coat after salting and allowed to ripen. As a result, salts and moisture diffused until uniform concentrations throughout the cheese mass were reached.

Figure 1 and 2 show chloride concentrations during ripening for cheeses S and K, respectively. Brines were prepared to obtain similar solute concentrations in the cheese aqueous phase during ripening since NaCl and KCl uptake rates are different (Zorrilla and Rubiolo 1994). Similar chloride profiles were obtained for cheeses salted with NaCl or NaCl/KCl mixture. Chloride content at the central zone increased while at the external zone it decreased with ripening time to reach approximately an average value of 0.9 g / 100 g cheese for both cheeses S and K at about 30 d. Analogous moisture mobility during ripening was observed for cheeses S and K (Figure 3 and 4). Moisture content at the central zone decreased while at the external zone it increased with ripening time to reach approximately an average value of 47 g/100 g cheese for both cheeses S and K at about 30 d. The behavior of salt and moisture profiles obtained are in agreement with studies related to partial substitution of NaCl by KCl in Fynbo cheese (Zorrilla 1993; Laborda 2000).

Considering the cheese as a homogeneous solid that does not change its structure during ripening, it is expected that effective diffusion coefficient increases with temperature (Turhan and Kaletunc 1992), increasing the diffusion rate. However, ripening temperature also affects composition of the cheese microflora, the rate of proteolysis, and the texture (Folkertsma and others 1996), modifying cheese structure. Therefore, effective pores and tortuosity may change, influencing the effective diffusion coefficient. Our

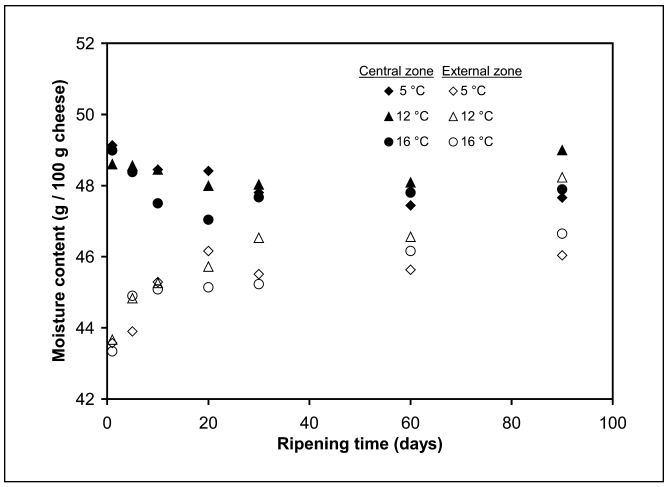

Figure 3—Profiles of moisture content during ripening of Fynbo cheeses salted with NaCl

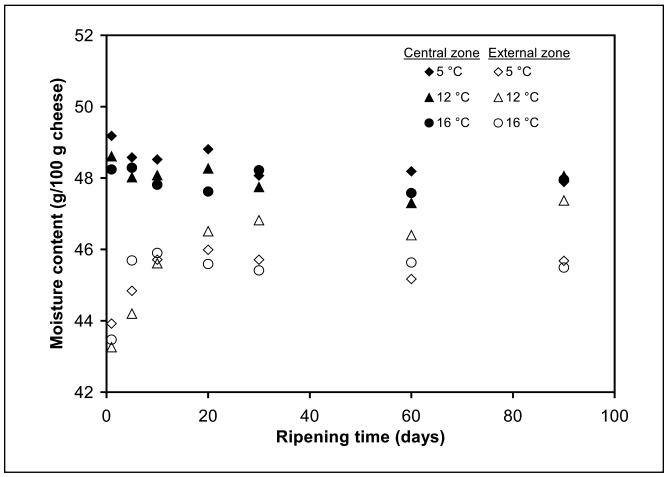

Figure 4—Profiles of moisture content during ripening of Fynbo cheeses salted with the mixture NaCl/KCl

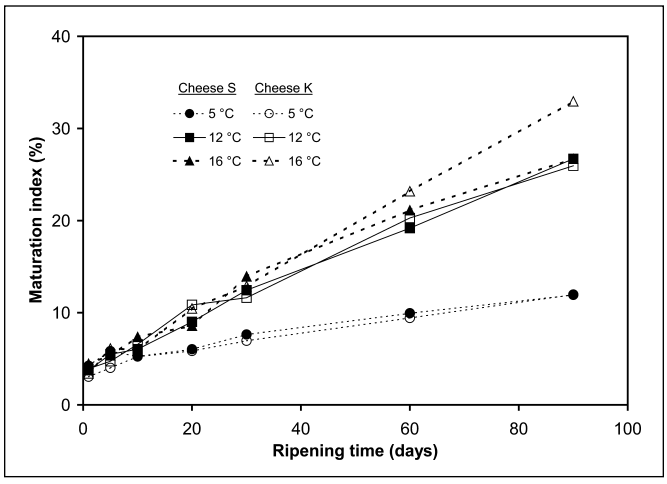

Figure 5—Maturation index changing with ripening time for the central zone in Fynbo cheeses

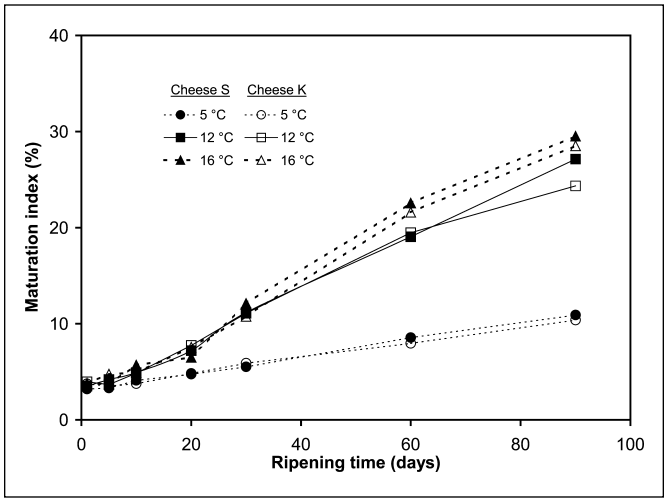

Figure 6—Maturation index changing with ripening time for the external zone in Fynbo cheeses

results showed that different temperatures during ripening did not strongly affect chloride distribution (Figure 1 and 2). Diffusion rate was slightly slower at 5 °C during the 1st 10 d for both sampling zones and cheeses studied. Moreover, moisture contents were not affected by temperature in the cases studied (Figure 3 and 4).

Maturation index

Figure 5 and 6 show the maturation index values during aging for central and external zones, respectively. Maturation index values, which are related to cheese proteolysis, increased with ripening time as expected.

Salt influences proteolysis through its effect on bacterial growth and enzyme activities. The residual rennet action on the 1st stage of proteolysis is particularly important in cheeses produced from casein coagulation by rennet as occurred for Fynbo cheeses. Noomen (1978) studied the proteolytic activity of rennet in simulated soft cheeses under various conditions by the determination of nitrogen soluble in the cheese moisture and by polyacrylamide gel electrophoresis. The soluble nitrogen values indicated that the total breakdown of protein was maximal in the absence of NaCl. Nevertheless, similar studies when NaCl was partially replaced by KCl are scarce.

The effects of NaCl and KCl on the activity of thermophilic lactic natural starters (milk starters and whey starters), used for cheese production in Argentina, and thermophilic lactic acid bacteria were investigated by Reinheimer and others (1997). High salt levels (4%) resulted in growth inhibition. It was noted that the salt type did not influence the residual acidifying and proteolytic activities. Moreover, they concluded that replacement of NaCl by KCl did not produce significant changes in the performance of thermophilic lactic acid bacteria. These results are in agreement with those obtained by Reddy and Marth (1995), who observed that counts of aerobic microorganisms, lactic acid bacteria, nonstarter lactic acid bacteria, aerobic spores, coliforms, yeasts, and molds in Cheddar cheeses made with KCl or NaCl/KCl mixtures were not significantly different from those of the control cheeses. However, there is relatively little information about the influence of NaCl or KCl on microbial enzymes in cheese.

In this work, no significant differences between IM values were observed due to the salt type used. Iwanczak and others (1995) studied the proteolysis of different cheese varieties salted with NaCl and NaCl/KCl mixtures, determining the content of pH 4.6 soluble nitrogen, nonprotein nitrogen, and peptide nitrogen. They concluded that KCl did not affect cheese proteolysis. Similarly, Laborda (2000) found that maturation index during ripening of Fynbo cheese was not affected by the type of salting procedure (NaCl or NaCl/KCl mixture). On the other hand, only few samples showed a significant difference in IM values due to sampling zone, being higher for the central than for the external zone. Laborda (2000) also studied Fynbo cheese proteolysis at central and external zones and concluded that sampling zone did not affect maturation index. However, the positions in cheese chosen for sampling in that case had a smaller salt concentration difference than in the present work.

Temperature is a major factor that affects bacterial growth and enzyme activities during manufacture and ripening of cheeses. Shakeel-Ur-Rehman and others (2000) studied the effect of ripening temperature on the growth and significance of nonstarter lactic acid bacteria (NSLAB) in Cheddar cheese made from raw or pasteurized milk, and the role of NSLAB in cheese biochemistry and flavor. They observed differences in the number of NSLAB of cheeses ripened at 1 and 8 °C. The maximum differences occurred at 4 mo, when the number of NSLAB in cheeses ripened at 8 °C were 3 log cycles higher than in those ripened at 1 °C. A contribution of NSLAB to the formation of free amino acids was established. Moreover, WSN was higher in cheeses ripened at 8 °C, reflecting the effect of higher temperature on the activity of chymosin and plasmin, which are primarily responsible for the production of WSN. Laborda and Rubiolo (1999) found that Fynbo cheeses stored at 16 °C showed higher level of WSN/TN than cheeses ripened at 12 °C. Moreover, the chromatographic method (RP-HPLC analysis of WSN fraction) showed that the higher temperature influenced mainly the formation of hydrophobic peptides. Our results showed that the production rate of soluble peptides increased as ripening temperature increased. The difference was more evident after 30 d and when IM values in Fynbo cheeses ripened at 5 °C were compared with those of cheeses ripened at 12 or 16 °C.

Electrophoretic analysis

Polyacrylamide gel electrophoresis (PAGE) has been increasingly used as a method to study casein hydrolysis and to follow cheese proteolysis. Marcos and others (1979) proposed a basic electrophoretic pattern common to most European cheeses made from cow milk. Furthermore, some results of primary proteolysis in solution obtained by PAGE have been extrapolated to cheeses (McSweeney and others 1994). These authors reported that most of the proteolysis in Cheddar cheese detectable by urea-PAGE could be explained by the action of chymosin, plasmin, and pepsin and also indicated that the major cleavage sites (in solution) of chymosin on α_{s1}-casein and of plasmin on β-casein are hydrolyzed in cheese.

A urea-PAGE electrophoretogram of samples obtained from Fynbo cheeses is shown in Figure 7. Slots 1 and 6 show β- and α_{s1}-casein standards, respectively. Standards allowed identifying the α_{s1}- and β-casein fractions by mobility comparison. Other electrophoretic fractions were assigned considering previous works (Marcos and others 1979, McSweeney and others 1994), such as γ-caseins and α_{s1}-I-casein. The γ-caseins (γ_1, γ_2, and γ_3) are produced from β-casein by the action of plasmin, while α_{s1}-I-casein is the primary product of rennet action on α_{s1}-casein arising from cleavage of Phe^{23}-Phe^{24}. Moreover, α_{s1}-I-casein seemed to be the unique

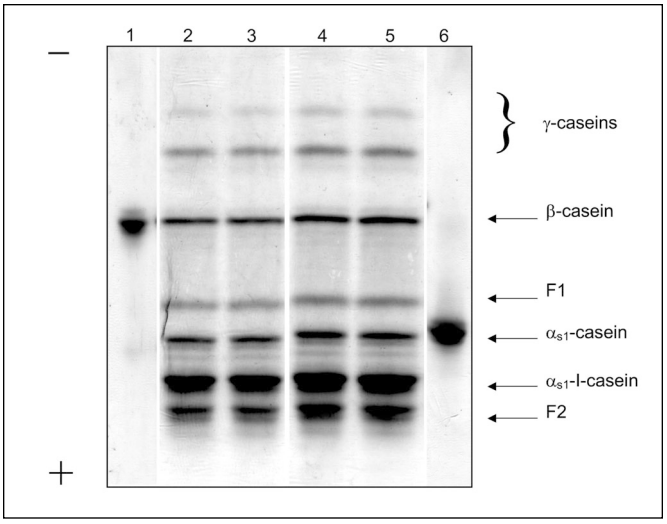

Figure 7—Urea-polyacrylamide gel electrophoretogram for Fynbo cheeses. Slot 1: β-casein standard; slots 2 and 3: patterns for a sample of a cheese S, 16 °C ripening temperature, central zone, and 90 d of ripening; slots 4 and 5: patterns for a sample of a cheese K, 16 °C ripening temperature, central zone, and 90 d of ripening; slot 6: α_{s1}-casein standard.

Table 1—Relative areas (%) for the different casein fractions for cheeses salted with NaCl[a].

Temp (°C)	Cheese zone	Ripening time (d)	γ	β	F1	α_{s1}	α_{s1}–I	F2
5	Central	1	1.41 ± 0.49	43.80 ± 3.06	0.77 ± 0.04	53.61 ± 3.15	0.39 ± 0.56	ND[b]
		5	3.85 ± 0.90	39.19 ± 4.28	ND	55.89 ± 2.96	1.12 ± 0.42	ND
		10	1.52 ± 0.11	41.36 ± 1.89	1.79 ± 0.43	52.89 ± 1.70	2.43 ± 0.14	ND
		20	1.60 ± 0.08	42.43 ± 1.08	2.85 ± 0.28	48.00 ± 1.01	5.09 ± 0.25	ND
		30	0.44 ± 0.63	38.49 ± 1.78	0.79 ± 1.12	52.60 ± 0.37	7.67 ± 0.35	ND
		60	2.16 ± 0.38	34.73 ± 1.01	2.32 ± 0.38	44.41 ± 0.79	15.57 ± 0.21	0.81 ± 0.01
		90	2.84 ± 0.93	30.11 ± 0.37	2.85 ± 0.40	43.14 ± 1.81	20.42 ± 0.18	0.62 ± 0.07
	External	1	2.56 ± 0.16	41.55 ± 0.54	0.56 ± 0.09	54.61 ± 0.33	0.69 ± 0.13	ND
		5	1.59 ± 0.42	41.97 ± 0.33	0.63 ± 0.28	54.68 ± 0.36	1.10 ± 0.01	ND
		10	0.95 ± 0.07	43.00 ± 0.65	0.48 ± 0.68	53.56 ± 0.08	2.00 ± 0.13	ND
		20	1.37 ± 0.13	42.59 ± 0.03	ND	52.37 ± 0.06	3.81 ± 0.18	ND
		30	ND	43.11 ± 0.07	ND	53.02 ± 0.11	3.86 ± 0.18	ND
		60	1.25 ± 0.20	37.65 ± 0.26	2.11 ± 0.21	44.88 ± 0.80	13.50± 0.24	0.59 ± 0.40
		90	3.34 ± 0.24	25.90 ± 0.95	2.65 ± 0.37	47.84 ± 0.25	19.64 ± 0.02	0.62 ± 0.58
12	Central	1	ND	42.60 ± 0.38	ND	56.92 ± 1.05	0.47 ± 0.67	ND
		5	ND	38.61 ± 2.09	ND	58.43 ± 1.16	2.95 ± 0.94	ND
		10	ND	37.10 ± 3.41	0.51 ± 0.73	56.01 ± 0.56	6.37 ± 2.12	ND
		20	0.75 ± 1.06	32.56 ± 0.59	0.69 ± 0.18	50.42 ± 0.52	15.70 ± 0.01	ND
		30	4.21 ± 0.35	34.78 ± 2.11	1.10 ± 0.19	37.02 ± 2.08	22.11 ± 0.19	0.77 ± 0.00
		60	5.28 ± 0.26	23.44 ± 0.84	1.85 ± 0.70	27.97 ± 1.22	37.55 ± 0.85	3.89 ± 1.93
		90	7.38 ± 0.61	18.23 ± 0.30	2.70 ± 0.37	18.54 ± 0.13	42.97 ± 0.20	10.17 ± 0.01
	External	1	ND	29.02 ± 2.75	ND	69.65 ± 3.85	1.33 ± 1.09	ND
		5	1.69± 2.39	36.68 ± 0.62	0.64 ± 0.91	58.93 ± 2.85	2.05 ± 0.17	ND
		10	ND	30.53 ± 0.93	ND	65.09± 0.97	4.20 ± 0.23	ND
		20	2.20 ± 0.93	32.06 ± 0.03	2.32 ± 0.07	51.31 ± 0.78	12.11 ± 0.89	ND
		30	5.08 ± 0.47	32.52 ± 1.43	2.14 ± 0.35	44.97 ± 0.00	14.20 ± 1.48	1.07 ± 0.17
		60	5.43 ± 1.03	13.53 ± 0.13	1.67 ± 2.37	46.58 ± 2.14	25.29 ± 1.28	7.49 ± 2.21
		90	8.61 ± 0.28	9.36 ± 0.39	ND	33.69 ± 0.56	31.56 ± 0.52	16.77 ± 1.19
16	Central	1	0.93 ± 0.21	39.72 ± 1.69	1.22 ± 0.42	57.30 ± 1.42	0.78 ± 0.06	ND
		5	ND	39.90 ± 0.43	2.51 ± 0.64	48.97 ± 0.74	8.58 ± 0.93	ND
		10	ND	36.87 ± 1.65	ND	50.00 ± 0.00	13.11 ± 1.65	ND
		20	2.09 ± 0.01	30.99 ± 2.06	ND	47.35 ± 1.21	19.55 ± 0.85	ND
		30	4.10 ± 0.42	31.34 ± 0.01	1.93 ± 0.51	37.91 ± 0.28	24.68 ± 0.63	ND
		60	7.37 ± 0.06	22.00 ± 3.07	4.25 ± 0.21	22.49 ± 0.50	38.43 ± 3.08	5.44 ± 0.25
		90	8.70 ± 1.10	12.26 ± 1.20	7.80 ± 0.95	14.68 ± 0.52	41.71 ± 0.68	14.84 ± 1.21
	External	1	0.73 ± 0.29	36.73 ± 0.40	1.04 ± 0.11	60.94 ± 0.08	0.55 ± 0.06	ND
		5	ND	40.16 ± 0.95	2.93 ± 0.61	48.95 ± 0.30	7.95 ± 0.64	ND
		10	ND	38.60 ± 0.47	ND	50.04 ± 0.81	11.34 ± 0.33	ND
		20	2.12 ± 0.36	27.57 ± 0.83	1.55 ± 0.62	56.20 ± 1.25	12.56 ± 1.36	ND
		30	5.13 ± 0.98	28.57 ± 1.44	1.87 ± 0.56	41.48 ± 0.80	22.92 ± 1.82	ND
		60	9.46 ± 2.99	14.60 ± 1.18	4.84 ± 2.67	35.13 ± 0.74	26.53 ± 0.78	9.41 ± 0.81
		90	14.72 ± 0.82	7.92 ± 0.28	11.44 ± 0.37	25.45 ± 1.17	24.37 ± 0.32	16.08 ± 0.56

[a]Mean values ± standard deviation. Initial casein fractions: α_{s1} (55.66%), β (44.34%).
[b]ND: not detected.

product of α_{s1}-casein that could be identified without ambiguity in cheese PAGE patterns (Marcos and others 1979). McSweeney and others (1994) showed that all of the peptides obtained from α_{s1}-casein during the ripening of Cheddar cheese had higher mobilities than α_{s1}-casein. Furthermore, similar results had been obtained by McSweeney and others (1993) when the proteolytic specificity of chymosin on α_{s1}-casein in solution and under different conditions was studied. Thus, F1 may be a product of β-casein degradation. On the other hand, several peptides, which are products of α_{s1}- or α_{s1}–I-casein degradation, have higher mobilities than α_{s1}-casein (McSweeney and others 1994). Therefore, F2 may be associated with those products.

Table 1 and 2 show the casein fractions determined as a relative area of the peaks obtained from electrophoretogram scanning for cheeses S and K, respectively. The quantitative analysis shows that α_{s1} and β fractions generally decreased, while F1, F2, α_{s1}–I, and γ fractions generally increased during ripening. The residual coagulant action in the 1st stage of proteolysis is evident because the α_{s1}-casein degradation was more pronounced during the 1st 30 d compared to β-casein degradation. After 30 d of ripening, β-casein degradation may be related to the production of F1 fraction because of the residual coagulant action and to the production of γ fraction because of the plasmin action.

Results obtained from the urea-PAGE patterns of cheeses S and K during maturation were not significantly different for any ripening temperature, suggesting that the casein hydrolysis was not influenced by the type of salt used. Katsiari and others (2000) compared proteolysis in Feta cheeses salted with mixtures of NaCl and KCl to that of control cheeses salted with NaCl and reported that there were no significant differences in the degradation of α_{s1}- and β- caseins at any sampling ages. Furthermore, Katsiari and others (2001) observed similar PAGE patterns for control Kefalograviera cheeses compared to those salted with NaCl/KCl mixtures, suggesting that the mode and rate of casein breakdown were similar in all cheeses.

Significant differences in casein degradation between central and external zones were observed (Table 1 and 2). The effect of salt concentration on the extent of proteolysis of α_{s1}- and β-casein in Cheddar cheese was demonstrated by Thomas and Pearce (1981). After 1 mo of storage at 10 °C, α_{s1}-casein had been almost complete-

Table 2—Relative areas (%) for the different casein fractions for cheeses salted with the NaCl/KCl mixture[a]

Temp (°C)	Cheese zone	Ripening time (d)	γ	β	F1	α_{s1}	α_{s1}–I	F2
5	Central	1	3.53 ± 0.01	42.62 ± 1.38	0.93 ± 0.10	51.45 ± 1.99	1.44 ± 0.51	ND[b]
		5	0.36 ± 0.52	43.43 ± 6.14	1.12 ± 0.09	52.89 ± 6.34	2.17 ± 0.80	ND
		10	0.60 ± 0.85	42.43 ± 0.18	1.66 ± 0.12	52.35 ± 0.04	2.95 ± 0.50	ND
		20	2.29 ± 0.55	42.02 ± 1.21	2.64 ± 0.65	47.78 ± 1.24	5.26 ± 0.07	ND
		30	1.43 ± 0.08	36.90 ± 0.42	1.93 ± 0.10	50.48 ± 0.79	9.25 ± 1.05	ND
		60	2.24 ± 0.08	36.28 ± 0.28	1.74 ± 0.52	43.48 ± 0.49	14.93 ± 0.12	1.31 ± 0.22
		90	2.68 ± 0.47	28.87 ± 1.45	2.74 ± 0.11	43.09 ± 1.73	21.80 ± 1.16	0.70 ± 0.69
	External	1	1.61 ± 0.30	44.24 ± 1.90	0.58 ± 0.03	52.96 ± 1.44	0.57 ± 0.11	ND
		5	6.97 ± 0.39	38.45 ± 2.47	ND	54.57 ± 2.85	ND	ND
		10	ND	39.05 ± 4.66	1.38 ± 1.92	57.46 ± 2.52	2.12 ± 0.21	ND
		20	1.09 ± 0.32	41.23 ± 1.10	0.99 ± 1.40	53.14 ± 0.15	3.54 ± 0.79	ND
		30	ND	37.75 ± 0.92	1.35 ± 0.49	54.24 ± 0.41	6.65 ± 0.04	ND
		60	1.19 ± 0.09	35.95 ± 2.23	1.85 ± 0.56	50.01 ± 1.88	10.98 ± 0.12	ND
		90	3.00 ± 0.06	25.88 ± 1.66	3.09 ± 0.37	48.82 ± 0.08	18.71 ± 1.09	0.50 ± 0.04
12	Central	1	ND	33.34 ± 2.01	ND	64.92 ± 2.49	1.74 ± 0.47	ND
		5	ND	37.89 ± 0.32	ND	58.77 ± 0.61	3.33 ± 0.28	ND
		10	ND	30.32 ± 2.82	1.53 ± 0.73	60.98 ± 3.00	7.16 ± 0.56	ND
		20	1.86 ± 0.36	29.49 ± 0.27	1.85 ± 0.22	50.13 ± 0.93	16.66 ± 0.08	ND
		30	5.55 ± 0.03	30.40 ± 0.06	2.57 ± 0.57	36.17 ± 2.28	23.72 ± 2.74	1.68 ± 0.06
		60	6.74 ± 0.04	22.55 ± 0.27	ND	26.78 ± 0.06	39.72 ± 0.80	4.19 ± 0.42
		90	7.65 ± 0.57	16.54 ± 1.94	0.90 ± 1.28	19.21 ± 0.49	45.87 ± 0.30	9.82 ± 0.28
	External	1	ND	34.63 ± 0.98	ND	65.36 ± 0.98	ND	ND
		5	0.41 ± 0.59	40.14 ± 3.40	0.69 ± 0.98	56.78 ± 4.57	1.97 ± 0.40	ND
		10	1.11 ± 0.22	29.02 ± 0.16	ND	64.80 ± 0.73	5.04 ± 0.34	ND
		20	1.33 ± 0.04	30.25 ± 2.33	2.07 ± 0.26	53.77 ± 1.89	12.56 ± 0.74	ND
		30	4.13 ± 0.37	33.70 ± 1.11	ND	42.46 ± 0.81	18.55 ± 0.66	1.14 ± 0.59
		60	5.41± 1.88	15.86 ± 1.75	ND	48.19 ± 2.57	30.58 ± 2.37	ND
		90	7.73 ± 0.01	11.02 ± 0.01	ND	38.15 ± 0.01	27.98 ± 0.01	15.11 ± 0.01
16	Central	1	1.09 ± 0.56	44.90 ± 1.23	2.90 ± 0.11	49.62 ± 0.53	1.47 ± 1.09	ND
		5	ND	40.78 ± 4.29	ND	55.48 ± 3.78	3.72 ± 0.50	ND
		10	ND	39.22 ± 0.24	ND	49.50 ± 0.02	11.28 ± 0.24	ND
		20	1.84 ± 0.45	30.45 ± 0.16	ND	58.57 ± 0.89	9.15 ± 1.15	ND
		30	3.68 ± 0.11	31.14 ± 1.41	1.13 ± 0.12	35.84 ± 0.61	28.19 ± 0.80	ND
		60	8.60 ± 2.57	23.01 ± 1.50	3.66 ± 0.74	21.85 ± 0.33	37.39 ± 0.86	5.48 ± 0.21
		90	9.72 ± 0.74	11.27 ± 0.35	9.03 ± 0.79	15.22 ± 0.11	38.52 ± 0.01	16.23 ± 0.19
	External	1	0.78 ± 0.08	43.41 ± 3.16	2.29 ± 0.86	53.15 ± 4.45	0.73 ± 0.01	ND
		5	ND	42.90 ± 0.54	ND	52.86 ± 0.16	4.22 ± 0.70	ND
		10	ND	39.32 ± 0.27	ND	51.47 ± 0.54	9.21 ± 0.81	ND
		20	ND	32.79 ± 2.00	ND	64.78 ± 1.53	2.42 ± 0.47	ND
		30	3.08 ± 0.71	31.41 ± 1.60	1.14 ± 0.01	45.02 ± 0.90	19.33 ± 1.77	ND
		60	10.06 ± 1.04	15.52 ± 1.14	4.58 ± 0.87	39.86 ± 0.08	23.62 ± 0.40	6.34± 1.61
		90	11.12 ± 0.85	8.83 ± 0.64	12.15 ± 0.81	27.63 ± 0.91	25.98 ± 1.11	14.29 ± 0.88

[a]Mean values ± standard deviation. Initial casein fractions: α_{s1} (55.66%), β (44.34%).
[b]ND: not detected.

Table 3—Kinetics constants of α_{s1}-casein degradation, correlation coefficients (r), and standard errors (SE) using Eq. (1)

Cheese	Temperature	Central zone			External zone		
	(°C)	k (10^{-2} day^{-1})	r	SE (10^{-2})	k (10^{-2} day^{-1})	r	SE (10^{-2})
S	5	0.277	0.907	0.058	0.196	0.856	0.053
	12	1.327	0.990	0.083	0.700	0.922	0.131
	16	1.524	0.993	0.081	0.878	0.953	0.124
K	5	0.242	0.922	0.045	0.140	0.835	0.041
	12	1.403	0.985	0.108	0.533	0.852	0.146
	16	1.519	0.961	0.195	0.747	0.895	0.167

ly degraded in a zone where the salt in moisture was 4%, whereas only 40% had been hydrolyzed in a zone where salt in moisture was 8%. Similarly, β-casein was degraded up to 50% in the zone were the salt in moisture was 4% and 10% in the zone were salt in moisture was 8%. Moreover, Noomen (1978) found that the degradation of α_{s1}-casein was stimulated by NaCl concentrations in the moisture up to about 4% and retarded by higher salt contents, while β-casein degradation was maximal in the absence of NaCl. In this case, α_{s1}-casein degradation was higher at the central zone during maturation, as expected. However, β-casein degradation was slightly smaller at the central zone at the end of the ripening period studied.

The rate of hydrolysis of the different casein fractions strongly depends on the ripening temperature (Table 3 and 4). Both α_{s1}-

Table 4—Values for activation energy (Ea) of α_{s1}-casein degradation, correlation coefficients (r), and standard errors (SE) using Eq. (2).

	Central zone			External zone		
Cheese	Ea (kcal/gmol)	r	SE	Ea (kcal/gmol)	r	SE
S	26.06	0.960	7.62	22.63	0.976	5.00
K	28.24	0.950	9.33	25.02	0.986	4.18

and β-casein were more extensively degraded at higher ripening temperature.

Kinetics analysis

Zorrilla and Rubiolo (1997) showed that the α_{s1}-casein degradation could be adequately described by 1st-order reaction kinetics as follows:

$$C/C_0 = e^{-k\,t} \quad (1)$$

where C is the α_{s1}-casein concentration at any ripening time t, C_o is the initial α_{s1}-casein concentration, and k is the kinetics constant. Table 3 shows the kinetics constants and correlation coefficients for the regressions of α_{s1}-casein values changing with ripening time. Although there was no significant effect of salting type, the corresponding kinetics constants are reported for the sake of completeness. Values of k for central zones were approximately 2 times higher than values for external zones. As expected, k values increased with ripening temperature. Values of k at 12 °C were in the order of those values obtained by Zorrilla and Rubiolo (1997) for Fynbo cheese.

The activation energy (Ea) for the reaction were calculated using the linearized Arrhenius equation:

$$\ln k = -Ea / RT + \ln A \quad (2)$$

where R is the gas constant (0.001987 kcal $gmol^{-1}$ K^{-1}), A is a constant called the frequency factor, and T is the ripening temperature (K). Table 4 shows the values of Ea for the different cheese zones and type of salt used, being significantly higher for central than for external zones. Laborda (2000) reported similar Ea value (20 kcal/gmol) for the formation of the pH 4.6 water-soluble nitrogen fraction during ripening of Fynbo cheeses.

Conclusions

THE EFFECT OF PARTIAL NACL REPLACEMENT BY KCL AND ripening temperature on Fynbo cheese proteolysis was studied. Cheeses salted with the mixture of NaCl/KCl had similar moisture and chloride contents and proteolysis than cheeses salted with NaCl. Chloride and moisture content profiles depended on the cheese sample position, but slightly depended on ripening temperature. Averaged values of 0.9 g chloride / 100 g cheese and 47 g moisture/100 g cheese were reached at 30 d of ripening. Maturation index changed with cheese zone, ripening time, and temperature. Six fractions obtained from urea-PAGE were studied: γ, β, α_{s1}, α_{s1}–I, and 2 unidentified fractions. The α_{s1}- and β-casein fractions decreased while the rest of the fractions increased during ripening. The α_{s1}-casein was more degraded at the central zone of the cheese. The β-casein was scarcely degraded during the 1st 30 d of ripening and it was slightly more degraded at the external zone of the cheese after 30 d of ripening. Ripening temperature increased casein degradation rate. The α_{s1}-casein degradation was studied through 1st-order reaction kinetics. Kinetics constants were in the range of 0.002 to 0.016 day^{-1} and the activation energy of the reaction was approximately 26 kcal/gmol in the range of 5 to 16 °C. The values obtained are useful information to characterize cheese proteolysis and to improve the control of Fynbo cheese processing.

References

Aly ME. 1995. An attempt for producing low-sodium Feta-type cheese. Food Chem 52:295-299.

Folkertsma B, Fox PF, McSweeney PLH. 1996. Accelerated ripening of Cheddar cheese at elevated temperatures. Int Dairy J 6:1117-1134.

Geurts TJ, Walstra P, Mulder H. 1972. Brine composition and the prevention of the defect "soft rind"in cheese. Neth Milk Dairy J 26:168-179.

Guinee TP, Fox PF. 1987. Salt in cheese: Physical, chemical and biological aspects. In: Fox PF, editor. Cheese: Chemistry, physics and microbiology. Volume 1. General aspects. London/New York: Elsevier Applied Science. P 251-297.

Innocente N, Corradini C. 1996. Effect of ripening temperature on the maturation of Montasio cheese. Ital J Food Sci 4:291-302.

Iwanczak M, Reps A, Wisniewska K, Jarmul I, Kolakowski P. 1995. Possibility for increasing the potassium content in ripening cheeses. Milchwissenschaft 50(11):619-622.

Katsiari MC, Voutsinas LP, Alichanidis E, Roussis IG. 1998. Manufacture of Kefalograviera cheese with less sodium by partial replacement of NaCl with KCl. Food Chem 61:63-70.

Katsiari MC, Alichanidis E, Voutsinas LP, Roussis IG. 2000. Proteolysis in reduced sodium Feta cheese made by partial substitution of NaCl by KCl. Int Dairy J 10:635-646.

Katsiari MC, Alichanidis E, Voutsinas LP, Roussis IG. 2001. Proteolysis in reduced sodium Kefalograviera cheese made by partial replacement of NaCl with KCl. Food Chem 73:31-43.

Kuchroo CN, Fox PF. 1982. Soluble nitrogen in Cheddar cheese: comparison of extraction procedures. Milchwissenschaft 37(6):331-335.

Laborda MA, Rubiolo AC. 1999. Proteolysis of Fynbo salted with NaCl/KCl and ripened at 2 temperatures. J Food Sci 64(1):33-36.

Laborda MA. 2000. Maduración de quesos con bajo contenido de cloruro de sodio [DPhil thesis]. Facultad de Ingeniería Química, Univ. Nacional del Litoral. Santa Fe, Argentina. 212 p. Available from: CERIDE Library, Güemes 3450, Santa Fe, Argentina.

Lesage L, Voilley A, Lorient D, Bezard J. 1993. Sodium chloride and magnesium chloride affected by ripening of Camembert cheese. J Food Sci 58:1303-1306.

Marcos A, Esteban MA, León F, Fernández-Salguero J. 1979. Electrophoretic patterns of European cheeses: Comparison and quantitation. J Dairy Sci 62:892-900.

McKenzie HA. 1971. Milk proteins. Chemistry and molecular biology. Volume 2. New York: Academic Press. 552 p.

McSweeney PLH, Olson NF, Fox PF, Healy A, Hojrup P. 1993. Proteolytic specificity of chymosin on bovine α_{s1}-casein. J Dairy Res 60:401-412.

McSweeney PLH, Pochet S, Fox PF, Healy A. 1994. Partial identification of peptides from the water-insoluble fraction of Cheddar cheese. J Dairy Res 61:587-590.

Noomen A. 1978. Activity of proteolytic enzymes in simulated soft cheeses (Meshanger type). 2. Activity of calf rennet. Neth Milk Dairy J 32:49-68.

Reddy KA, Marth EH. 1991. Reducing the sodium content of foods: a review. J Food Prot 54(2):138-150.

Reddy KA, Marth EH. 1993. Proteolysis in Cheddar cheese made with sodium chloride, potassium chloride or mixtures of sodium and potassium chloride. Lebensm-Wiss Technol 26:434-442.

Reddy KA, Marth EH. 1995. Microflora of Cheddar cheese made with sodium chloride, potassium chloride, or mixtures of sodium and potassium chloride. J Food Prot 58(1):54-61.

Reinheimer JA, Renzulli PM, Rubiolo AC, Bailo NB, Binetti AG. 1997. Effects of sodium chloride and potassium chloride on growth and acid production in thermophilic lactic acid bacteria. Microbiol Alim Nutr 15:7-15.

Shakeel-Ur-Rehman, Banks JM, Brechany EY, Muir DD, McSweeney PLH, Fox PF. 2000. Influence of ripening temperature on the volatiles profile and flavor of Cheddar cheese made from raw or pasteurised milk. Int Dairy J 10:55-65.

Thomas TD, Pearce KN. 1981. Influence of salt on lactose fermentation and proteolysis in Cheddar cheese. New Zealand Dairy Sci Technol 16:253-259.

Turhan M, Kaletunc G. 1992. Modeling of salt diffusion in white cheese during long-term brining. J Food Sci 57(5):1082-1085.

Zorrilla SE. 1993. Difusión de NaCl y KCl en quesos [DPhil thesis]. Facultad de Ingeniería Química, Univ. Nacional del Litoral. Santa Fe, Argentina. 228 p. Available from: CERIDE Library, Güemes 3450, Santa Fe, Argentina.

Zorrilla SE, Rubiolo AC. 1994. Fynbo cheese NaCl and KCl changes during ripening. J Food Sci 59(5):972-975.

Zorrilla SE, Rubiolo AC. 1997. Kinetics of casein degradation during ripening of Fynbo cheese salted with NaCl/KCl brine. J Food Sci 62(2):386-389.

Zorrilla SE, Rubiolo AC. 1999. Sensory analysis during ripening of Fynbo cheese salted with NaCl/KCl brine. Food Sci Tech Int 5(3):251-254.

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The authors are affiliated with Instituto de Desarrollo Tecnológico para la Industria Química (INTEC) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Univ. Nacional del Litoral (UNL) – Güemes 3450, (3000) Santa Fe, República Argentina. Direct inquiries to author Rubiolo (E-mail: arubiolo@intec.unl.edu.ar).