The effect of substituting NaCl with KCl on Nabulsi cheese: Chemical composition, total viable count, and texture profile

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ABSTRACT

The effect of substituting NaCl with KCl on Nabulsi cheese characteristics was investigated. Nabulsi cheese was made and stored in 4 different brine solutions at 18%, including NaCl only (A; control); 3NaCl:1KCl (wt/wt; B); 1NaCl:1KCl (wt/wt; C); and 1NaCl:3KCl (wt/wt; D). Chemical composition, proteolysis, total viable count, and texture profile analysis were assessed at monthly intervals for 5 mo. No significant effect was found among experimental cheeses in terms of chemical composition or texture profile. Proteolytic activities were higher in cheeses kept in brine solutions that contained higher KCl (B, C, and D) compared with the control. At the end of the storage period, water-soluble nitrogen in Nabulsi cheeses stored in B, C, and D was higher than that in the control cheese (A). In addition, total viable count increased significantly after 1 mo of storage for all salt treatments. Hardness and gumminess generally decreased significantly during storage within the same salt treatment.

Key words: Nabulsi cheese, proteolysis, texture profile, microstructure

INTRODUCTION

White brined cheeses are traditional cheeses of the Middle Eastern region: Halloumi cheese is popular in Cyprus and Lebanon, Domiati cheese in Egypt, and Nabulsi cheese in Jordan, Syria, and Palestine (Yamani et al., 1998). Nabulsi cheese is traditionally produced from sheep or goat milk, or both, during the spring and summer seasons. However, due to currently high demand, Nabulsi cheese is also produced from cow milk (Abu-Alruz et al., 2009b) or mixture a mixture of cow and sheep milk. It is classified as a semi-hard cheese with high salt content and produced without addition of starter culture (Abd El-Salam and Alichanidis, 2004; Abu-Alruz et al., 2009b). A brief procedure of Nabulsi cheese production is as follows: pasteurized

milk is tempered to approximately 35°C and rennet is added. After 30 to 60 min, the curd is cut and settled for 10 to 15 min, and then transferred into cheesecloth to drain whey; the curd is then pressed at 0.4 MPa, and the cheese is cut into small blocks ($\sim 4 \times 4 \times 1.5$ cm) and sprinkled with salt or immersed in a brine solution (18 to 21%) overnight. The cheese blocks are then boiled in a brine solution (18 to 21%) for 5 to 10 min, cooled, and stored in metal or plastic jars filled with a brine solution (Ibrahim and O'Sullivan, 1998; Abd El-Salam and Alichanidis, 2004; Tamime, 2006). Salt addition is a critical step during cheese making to maintain the quality and safety of Nabulsi cheese during 6 to 12 mo of storage. Nabulsi cheese is consumed as a part of breakfast or eaten as a snack and it is an essential component of the Middle Eastern sweets such as Kunafa (Yamani et al., 1998). Nabulsi cheese manufacturers intentionally increase the salt content of the cheese to promote water loss and to preserve cheese during prolonged storage.

Middle Eastern people may avoid consumption of Nabulsi cheese because of the high salt content. A positive correlation has been found between high sodium (Na) intake and hypertension (Kotchen, 2005), osteoporosis (Heaney, 2006), kidney stones (Massey, 2005), and cardiovascular diseases (Penner et al., 2007). The World Health Organization has recommended decreasing salt in all food types to reduce health problems associated with high intake of salt (World Health Organization, 2007). It has been reported that cheeses contribute to approximately 11 to 20% of the total sodium dietary intake (Guinee, 2004a,b; Tamime, 2006). Consequently, and because of high cheese consumption in Middle Eastern countries, Nabulsi cheese contributes to a considerable amount of the total dietary Na intake for consumers in the Middle East. Hence, it is important to reduce salt content in Nabulsi cheese rather than decreasing its consumption. Several studies have been carried out to improve the manufacturing procedure (Ibrahim and O'Sullivan, 1998), stretchability and meltability (Abu-Alruz et al., 2009b; Mazahreh et al., 2009), and the microbiological quality of Nabulsi cheese (Humied et al., 1990). This study is the first attempt aimed at reducing the salt content (NaCl) in Nabulsi

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cheese by substituting NaCl with KCl. In particular, the aim of this study was to investigate the effect of substituting NaCl with KCl on chemical composition, microbiological quality, proteolysis, and physical properties of Nabulsi cheese during storage at room temperature for 5 mo.

MATERIALS AND METHODS

Cheese Making

Nabulsi cheese was manufactured according to Abu-Alruz et al. (2009a) with some modifications as shown in Figure 1. Full-fat homogenized and pasteurized bovine milk was obtained from the local market (Melbourne, VIC, Australia). Forty liters of milk was tempered to 37°C, and diluted chymosin (Chy-Max, Chr. Hansen, Bayswater, VIC, Australia) was added according to the manufacturer's instruction. After 35 min, coagulated curd was cut into 1-cm³ cubes and left for 10 min. Then, the curd was transferred into cheesecloth and left for 15 min to drain the whey. The curd was pressed (each 1-kg weight of curd was pressed with 60 kg of weight) for 90 min and cut into 4 × 4 × 1.5 cm pieces, divided randomly and equally, and kept in 4 brine solutions (prepared using Milli-Q water; the pH of the brine solution was not adjusted) at 21%, including only NaCl (**A**, control), 3NaCl:1KCl (**B**), 1NaCl:1KCl (**C**), and 1NaCl:3KCl (**D**) and dipped for 72 h at room temperature (~21°C). Thereafter, each cheese portion was boiled for 15 min in the same brine solutions until the cheese temperature reached approximately 85°C, placed in clean and sanitized plastic containers, and cooled (~40°C). Finally, each batch of cheese was transferred into 4 fresh brine solutions at 18% in separate plastic containers and stored for 5 mo at room temperature (~21°C). Samples (~100 g each) were taken at monthly intervals to determine chemical composition, Na, K, Ca, and P contents, and enumeration of total viable count (**TVC**). All analyses were carried out in duplicate and all experiments were replicated 3 times.

Chemical Composition

Chemical composition was determined according to AOAC methods (AOAC, 1995); moisture was determined by oven-drying method at 102°C, fat by the Babcock method, protein by the Kjeldahl method, and ash by the muffle furnace method. For pH measurement, 20 g of grated cheese was macerated in 20 mL of distilled water, and the pH of the resultant slurry was measured using a calibrated digital pH meter (MeterLab, Pacific Laboratory Products, Melbourne, VIC, Australia).

TVC

Total viable count was enumerated according to an ISO/IDF method (ISO/IDF, 2002). Briefly, 11 g of grated cheese was blended with 99 mL of sterilized peptone and water solution (0.1% peptone) for 2 min using a Stomacher-400 laboratory blender (Seward Medical, London, UK). Appropriate dilutions were made using 0.1% peptone and water solution, and enumeration was carried out using tryptone soy agar (Oxoid Ltd., West Heidelberg, VIC, Australia). Plates were aerobically incubated at 37°C for 48 h.

Assessment of Proteolysis

The water-soluble extracts (**WSE**) of the cheese samples were prepared according to Kuchroo and Fox (1982). The nitrogen in the extract was estimated by the Kjeldahl method (AOAC, 1995). Twelve percent TCA-soluble nitrogen (**TCA-SN**) and 5% phosphotungstic acid (**PTA-SN**) were determined according to Ayyash and Shah (2010b). The TCA-SN was estimated in 9 mL of filtrate obtained after precipitation of filtered WSE of cheese with 24% TCA (Sigma-Aldrich, St. Louis, MO). The extent of secondary proteolysis (PTA-SN) was assayed similarly to TCA-SN using 9 mL of filtrate obtained after precipitation of filtered WSE of cheese with 10% phosphotungstic acid (Sigma-Aldrich).

Determination of Na, K, Ca, and P Contents

Contents of Na, K, Ca, and P in cheeses were determined by multitype inductively coupled plasma atomic emission spectrometry [ICPE-9000, Shimadzu Scientific Instruments (Oceania) Pty Ltd., Rydalmere, NSW, Australia] according to Ayyash and Shah (2010a). After the ash determination of cheese samples, an aliquot (~0.5 g) was dissolved in 10 mL of 1 N HNO₃ and kept frozen (-20°C) until analyzed using the ICPE-9000. All samples were diluted 100 times and then filtered using a Millex 0.45-μm filter (Millipore, Bedford, MA) before directly injecting into the ICPE-9000. The ICPE-9000 consisted of an ASC-6100 autosampler, a hydride generator HVG-ICP, a hydrofluoric acid sample injection system HFS-2, a low-temperature thermostatic chamber NCB-1200, and a software package ICPE-9000. To calculate Na, K, Ca, and P concentrations in samples, a standard curve consisting of the 4 elements was prepared at 1, 10, 20, 30, and 40 μg/mL.

Texture Profile Analysis

Texture profile (hardness, cohesiveness, adhesiveness, and gumminess) was analyzed according to Bhaska-

Figure 1. Procedure for manufacture of Nabulsi cheese.

acharya and Shah (1999) with some modifications. Cylinders (25 × 15 mm, diameter × height) of cheeses were cut from the center of the Nabulsi cheese blocks. Specimens were kept in a refrigerator at 4°C overnight

followed by determination of texture profile. Hardness, cohesiveness, adhesiveness, and gumminess were measured using an Instron universal testing machine (model 5564, Instron Ltd., London, UK) based on the principle

described by Pons and Fiszman (1996). Each sample was compressed to 50% of its height using a 100-N load cell with a flat plunger, and the crosshead movement was adjusted to 50 mm/min. A double compression was achieved and the data were collected using Merline software (Instron Pty Ltd. Melbourne, VIC, Australia). Analyses were carried out in duplicate.

Microstructure by Environmental Scanning Electron Microscopy

Experimental cheese specimens were imaged by using FEI Quanta environmental scanning electron microscopy (ESEM; Philips Electron Optics, Eindhoven, the Netherlands) using ESEM mode. Images were taken at accelerating voltage at 30 kV under 3.5 Torr of pressure and $1,200 \times$ magnification at 4°C. Specimens were not conductivity coated before imaging. Images were visually examined to determine differences among batches.

Statistical Analysis

One-way ANOVA was used to test differences among the 4 experimental cheeses (A, B, C, and D) at each salt treatment or sampling day, and significance was tested at $P < 0.05$. Pearson correlation was measured at $P < 0.05$ between all measured variables in the same salt treatment. Two-way ANOVA was carried out to investigate the effect of salt treatment and storage period interaction for all measured variables. Data were analyzed using SAS software version 9.2 (SAS Institute, 2008).

RESULTS AND DISCUSSION

Chemical Composition

Moisture, protein, fat, and ash contents and pH values of Nabulsi cheeses kept in 4 brine solution are presented in Table 1. In general, the moisture, protein, ash, and pH decreased significantly ($P < 0.05$) between 0 and 5 mo of storage within a salt treatment. However, storage period had no significant ($P > 0.05$) effect on the fat content (Table 1). The reduction in moisture content occurred due to the migration of moisture from cheese loaves as reported by Geurts et al. (1980) and Guinee and Fox (2004). The decrease in protein content may be attributed to the proteolytic activity during storage, which resulted in an increase in WSN and TCA-SN in experimental cheeses (Table 2). These findings agree with those of Ayyash and Shah (2010a), who reported that moisture and intact protein content of Halloumi cheese decreased ($P < 0.05$) during storage.

The decreases in ash content and pH are in contradiction with the results of Ayyash and Shah (2010a) and Katsiari et al. (1997, 1998). This may be attributed to the manufacturing process of Nabulsi cheese, which differs from those of Halloumi, Feta, and Kefalograviera cheeses. In this study, cheese loaves were dipped into brine solution at 21% for 72 h before boiling and storage. This step increased the salt content in cheeses, which was reflected in the ash content and pH value of cheese loaves before boiling and storing in brine solutions at a lower brine concentration of 18%. During storage, cheese loaves lost excessive salt to brine solution to reach an equilibrium, which in turn affected the ash content of cheeses. The decrease in pH values during storage may be due to microbial growth, especially that of nonstarter lactic acid bacteria, which may ferment lactose and produce organic acids in cheeses with decreased pH values.

In general, ANOVA showed no significant ($P < 0.05$) differences in chemical composition between experimental Nabulsi cheeses at the same storage times (Table 1). Occasional differences were observed between salt treatments in terms of moisture, protein, fat, and ash contents (Table 1). We assumed that these differences were due to variations in cheese loaves and did not relate to salt treatment.

As seen in Table 1, pH values of cheeses kept in the D brine solution were significantly higher compared with A (control) at 0 mo of storage. At 1 and 2 mo of storage, pH values of Nabulsi cheeses kept in D and C treatments were significantly higher compared with those of cheeses in the A and B treatments. This suggests that when KCl became a part of the brine solution, the pH values increased. This is due to the nature of KCl solutions, which have higher pH value (~ 0.3) compared with those of NaCl. This finding is in accordance with those of other researchers (Fitzgerald and Buckley, 1985; Katsiari et al., 1998; Ayyash and Shah, 2010a), who reported a slight increase in pH values of Kefalograviera, Halloumi, and Cheddar cheeses, respectively, made with NaCl-KCl mixtures. However, for the rest of the storage period, the pH values of cheeses kept in NaCl-KCl mixtures became significantly lower compared with the control cheeses (Table 1). This may be attributed to higher microbial activity in those cheeses because of high pH, which is more conducive to microbial growth (Ayyash and Shah, 2010a).

Assessment of Proteolysis and TVC

Water-soluble N, TCA-SN, PTA-SN, and TVC of Nabulsi cheeses kept in 4 brine solutions are presented in Table 2. Analysis of variance showed that TVC in-

Table 1. Moisture, protein, fat, and ash contents, and pH values of Nabulsi cheeses stored in 4 levels of NaCl and KCl during storage for 5 mo at room temperature¹

Storage (mo)	Salt treatment ²	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	pH
0	A	59.12 ± 1.32 ^a	25.21 ± 0.36 ^a	22.37 ± 0.94 ^a	10.88 ± 0.28 ^a	6.41 ± 0.02 ^b
	B	58.71 ± 1.18 ^a	24.76 ± 0.27 ^a	23.57 ± 0.23 ^a	10.12 ± 0.29 ^a	6.52 ± 0.03 ^b
	C	58.02 ± 0.69 ^{ab}	24.68 ± 1.63 ^a	23.93 ± 1.37 ^a	10.77 ± 0.17 ^a	6.50 ± 0.07 ^b
	D	55.25 ± 0.56 ^b	25.49 ± 0.58 ^a	23.50 ± 1.35 ^a	10.19 ± 0.20 ^a	6.65 ± 0.00 ^a
1	A	50.81 ± 0.34 ^a	21.81 ± 0.52 ^a	21.00 ± 0.06 ^a	10.22 ± 0.18 ^a	6.38 ± 0.09 ^c
	B	49.83 ± 0.85 ^{ab}	21.79 ± 0.25 ^a	21.00 ± 0.59 ^a	10.11 ± 0.21 ^a	6.55 ± 0.03 ^b
	C	50.01 ± 0.72 ^{ab}	21.79 ± 0.41 ^a	22.37 ± 0.47 ^a	10.06 ± 0.18 ^a	6.70 ± 0.01 ^a
	D	48.66 ± 0.25 ^b	21.58 ± 0.60 ^a	20.93 ± 0.78 ^a	9.92 ± 0.10 ^a	6.78 ± 0.01 ^a
2	A	51.67 ± 0.31 ^a	22.79 ± 0.66 ^a	22.20 ± 0.40 ^a	9.43 ± 0.66 ^a	7.00 ± 0.06 ^b
	B	50.80 ± 0.10 ^{ab}	22.26 ± 0.57 ^a	20.60 ± 0.32 ^b	9.74 ± 0.13 ^a	7.03 ± 0.04 ^b
	C	51.80 ± 0.47 ^a	23.49 ± 0.40 ^a	21.93 ± 0.61 ^{ab}	10.07 ± 0.25 ^a	7.20 ± 0.02 ^a
	D	49.44 ± 0.70 ^b	22.72 ± 0.98 ^a	22.63 ± 0.37 ^a	10.70 ± 0.18 ^a	7.23 ± 0.01 ^a
3	A	52.76 ± 1.45 ^a	23.19 ± 0.95 ^a	23.33 ± 0.27 ^a	9.98 ± 0.15 ^a	6.52 ± 0.03 ^a
	B	51.35 ± 0.41 ^a	21.74 ± 1.59 ^a	22.53 ± 0.32 ^{ab}	9.83 ± 0.10 ^a	6.41 ± 0.01 ^b
	C	52.59 ± 0.81 ^a	22.79 ± 0.52 ^a	22.70 ± 0.23 ^{ab}	10.04 ± 0.03 ^a	6.53 ± 0.00 ^a
	D	52.06 ± 0.49 ^a	22.12 ± 0.26 ^a	21.77 ± 0.64 ^b	9.78 ± 0.09 ^a	6.34 ± 0.01 ^c
4	A	51.54 ± 0.57 ^{ab}	23.17 ± 0.28 ^a	22.37 ± 0.13 ^a	9.90 ± 0.23 ^a	6.34 ± 0.00 ^a
	B	49.92 ± 0.36 ^{bc}	22.78 ± 0.78 ^a	22.50 ± 0.58 ^a	10.04 ± 0.02 ^a	6.10 ± 0.01 ^d
	C	51.87 ± 0.51 ^a	23.29 ± 0.25 ^a	23.33 ± 0.17 ^a	10.29 ± 0.05 ^a	6.26 ± 0.01 ^b
	D	49.46 ± 0.60 ^c	21.05 ± 0.39 ^b	22.33 ± 0.44 ^a	10.33 ± 0.19 ^a	6.20 ± 0.01 ^c
5	A	51.78 ± 0.39 ^a	22.72 ± 0.48 ^{ab}	21.97 ± 0.74 ^a	9.88 ± 0.16 ^b	6.49 ± 0.03 ^a
	B	49.72 ± 0.60 ^{ab}	22.03 ± 0.11 ^{bc}	21.07 ± 0.30 ^{ab}	10.10 ± 0.01 ^{ab}	6.16 ± 0.02 ^d
	C	50.93 ± 0.24 ^a	23.65 ± 0.13 ^a	21.73 ± 0.15 ^a	10.11 ± 0.02 ^{ab}	6.36 ± 0.01 ^b
	D	47.29 ± 1.43 ^b	21.38 ± 0.59 ^c	20.37 ± 0.19 ^b	10.22 ± 0.10 ^a	6.27 ± 0.01 ^c

^{a-d}Means in each column and at the same storage time with same letter did not differ significantly ($P > 0.05$).

¹Mean values ± SE of 3 trials.

²Salt treatment: A = NaCl only (control); B = 3NaCl:1KCl (wt/wt); C = 1NaCl:1KCl (wt/wt); D = 1NaCl:3KCl (wt/wt).

creased significantly ($P < 0.05$) from 0 to 3 mo of storage, whereas WSN, TCA-SN, and PTA-SN increased significantly ($P < 0.05$) in all experimental cheeses during 5 mo of storage. It was noticed that the WSN, TCA-SN, and PTA-SN contents in all experimental cheeses were lower in respect to the long storage period. This may due to the high heat treatment applied to cheeses during manufacturing. Heating experimental cheeses also inactivated the chymosin residues; chymosin is considered as a primary proteolytic agent in cheeses (Fox and McSweeney, 1996; Upadhyay et al., 2004). Thus, we assumed that plasmin (a heat-stable indigenous enzyme) remained in cheeses and acted as a primary proteolytic agent instead of chymosin. The activity of plasmin is slower than that of chymosin (Farkye and Fox, 1991; Fox and McSweeney, 1996; Upadhyay et al., 2004). Hence, the increase in WSN and TCA-SN was slower.

As shown in Table 2, in general, significant ($P < 0.05$) differences were observed among experimental cheeses in WSN at the same time of storage. Those differences were observed clearly in the latter part (3, 4, and 5 mo) of the storage period. It was apparent that WSN in cheeses stored in treatment D was higher ($P < 0.05$) compared with that in the other experimental cheeses at 4 and 5 mo of storage. This may suggest that plasmin activity increased in parallel with the increase

in KCl concentration. This finding is in accordance with Ayyash and Shah (2010b), who showed higher WSN values in experimental Halloumi cheeses stored in 1NaCl:1KCl and in 1NaCl:3KCl compared with control cheese during storage.

No significant ($P > 0.05$) differences in TCA-SN and PTA-SN were observed among experimental cheeses in the early part of the storage period (0, 1, and 2 mo). However, a significant ($P < 0.05$) difference was obvious at mo 5 of storage, where cheese in the D treatment showed higher ($P < 0.05$) TCA-SN compared with other cheeses. That may be attributed to the higher WSN of cheese in D treatment that, in turn, provided substrates (large and medium peptides) for spoilage microorganisms and finally increased TCA-SN. Upadhyay et al. (2004) reported that enzymes from starter culture and nonstarter bacteria hydrolyze peptides resulting from primary (chymosin) enzymatic activity. Significant ($P < 0.05$) differences were observed between experimental cheeses in TVC during mo 1 and 2. This suggests that NaCl substitution with KCl has a similar effect on TVC of Nabulsi cheese. This finding in agreement with those of Ayyash and Shah (2010b).

Water-soluble N, TCA-SN, and PTA-SN were inversely correlated with Ca and P contents in all salting treatments. Pearson correlations (r) of proteolysis variables ranged from -0.44 to -0.68 for Ca and from

Table 2. Water-soluble N (WSN), TCA-soluble N (TCA-SN), phosphotungstic acid-soluble N (PTA-SN), and total viable count (TVC) of Nabulsi cheeses stored in 4 levels of NaCl and KCl during storage for 5 mo at room temperature¹

Storage (mo)	Salt treatment ²	Parameter ³			
		WSN	TCA-SN	PTA-SN	TVC
0	A	1.02 ± 0.02 ^b	0.79 ± 0.04 ^a	0.39 ± 0.02 ^a	ND ⁴
	B	1.32 ± 0.12 ^a	0.81 ± 0.03 ^a	0.33 ± 0.04 ^a	ND
	C	1.09 ± 0.09 ^{ab}	0.87 ± 0.04 ^a	0.39 ± 0.01 ^a	ND
	D	1.10 ± 0.07 ^{ab}	0.83 ± 0.01 ^a	0.33 ± 0.01 ^a	ND
1	A	1.04 ± 0.10 ^a	0.34 ± 0.05 ^a	0.16 ± 0.05 ^a	4.56 ± 0.01 ^a
	B	1.02 ± 0.01 ^a	0.45 ± 0.06 ^a	0.17 ± 0.03 ^a	4.11 ± 0.18 ^b
	C	1.12 ± 0.08 ^a	0.38 ± 0.01 ^a	0.16 ± 0.00 ^a	4.37 ± 0.10 ^{ab}
	D	0.96 ± 0.02 ^a	0.39 ± 0.06 ^a	0.20 ± 0.04 ^a	4.34 ± 0.12 ^{ab}
2	A	1.26 ± 0.06 ^b	0.91 ± 0.04 ^a	0.34 ± 0.02 ^a	5.29 ± 0.10 ^a
	B	1.54 ± 0.09 ^{ab}	1.14 ± 0.27 ^a	0.29 ± 0.04 ^a	5.10 ± 0.10 ^{ab}
	C	1.53 ± 0.05 ^{ab}	0.75 ± 0.06 ^a	0.27 ± 0.03 ^a	4.79 ± 0.01 ^b
	D	1.57 ± 0.14 ^a	0.89 ± 0.06 ^a	0.31 ± 0.05 ^a	4.93 ± 0.14 ^b
3	A	2.18 ± 0.21 ^a	3.43 ± 0.44 ^a	1.20 ± 0.12 ^a	6.06 ± 0.18 ^a
	B	1.60 ± 0.18 ^b	1.10 ± 0.13 ^b	0.46 ± 0.06 ^b	5.62 ± 0.14 ^a
	C	1.43 ± 0.06 ^b	0.89 ± 0.05 ^b	0.27 ± 0.01 ^b	5.61 ± 0.17 ^a
	D	1.86 ± 0.06 ^{ab}	1.33 ± 0.14 ^b	0.26 ± 0.02 ^b	5.57 ± 0.16 ^a
4	A	1.78 ± 0.06 ^b	1.10 ± 0.55 ^a	0.77 ± 0.04 ^a	5.22 ± 0.04 ^a
	B	1.85 ± 0.02 ^b	1.16 ± 0.06 ^a	0.33 ± 0.02 ^b	5.00 ± 0.26 ^a
	C	1.94 ± 0.06 ^b	1.34 ± 0.13 ^a	0.37 ± 0.07 ^b	4.99 ± 0.17 ^a
	D	2.81 ± 0.07 ^a	2.09 ± 0.40 ^a	0.37 ± 0.19 ^b	4.80 ± 0.31 ^a
5	A	3.81 ± 0.15 ^c	4.38 ± 0.28 ^b	2.44 ± 0.06 ^a	5.20 ± 0.05 ^a
	B	4.63 ± 0.23 ^b	3.61 ± 0.12 ^c	1.15 ± 0.05 ^c	5.05 ± 0.24 ^a
	C	4.05 ± 0.14 ^{bc}	3.03 ± 0.05 ^d	0.69 ± 0.35 ^c	5.02 ± 0.16 ^a
	D	6.86 ± 0.35 ^a	5.31 ± 0.09 ^a	1.78 ± 0.10 ^b	4.93 ± 0.38 ^a

^{a-d}Means in each column and at the same storage time with same letter did not differ significantly ($P > 0.05$).

¹Mean values ± SE of 3 trials.

²Salt treatment: A = NaCl only (control); B = 3NaCl:1KCl (wt/wt); C = 1NaCl:1KCl (wt/wt); D = 1NaCl:3KCl (wt/wt).

³WSN = water-soluble N as a percentage of total N; TCA-SN = 12% TCA-soluble N as a percentage of total N; PTA-SN = 5% phosphotungstic acid-soluble N as a percentage of total N; TVC = total viable count ($\log_{10}$ cfu/g).

⁴ND = not detected.

−0.23 to −0.69 for P. This supports the suggestion that a reduction in colloidal calcium in cheeses increased proteolytic activity. In addition, TVC inversely was correlated with Ca and P in all salting treatments; r-values ranged from −0.65 to −0.79 for Ca and from −0.66 to −0.79 for P. This may be attributed to proteolysis: when Ca and P contents decreased during storage, proteolytic activity increased and then provided primary nutrients essential for microbial growth.

Texture Profile Analysis

Hardness, cohesiveness, adhesiveness, and gumminess of experimental cheeses are shown in Table 3. According to the ANOVA, adhesiveness did not change significantly ($P > 0.05$), whereas cohesiveness increased ($P < 0.05$) and hardness decreased ($P < 0.05$) during storage. This may due to increased proteolytic activity that reduced the protein network; hence, hardness decreased while cohesiveness and adhesiveness increased (Lawrence et al., 1987; Johnson and Lucey, 2006). In addition, reductions in Ca and P contents during stor-

age may have decreased cross-linkage between caseins, resulting in decreased hardness (Guinee et al., 2002; Joshi et al., 2003).

Although a slight difference in texture profile was observed between experimental cheeses at specific sampling times, no significant ($P > 0.05$) difference was observed among experimental cheeses during most of the storage period. This may be attributed to natural differences between cheeses loaves and not related to salting treatments. These findings are in accordance with those of Ayyash and Shah (2011) and Katsiari et al. (1997, 1998), who reported no significant ($P > 0.05$) differences between experimental cheeses in textural profile of Feta, Kefalograviera, and Halloumi cheeses.

Hardness and gumminess were positively correlated ($P < 0.05$) with Ca (r-values from 0.64 to 0.81 and 0.17 to 0.62, respectively) and P (from 0.63 to 0.80 and 0.37 to 0.53, respectively); however, cohesiveness and adhesiveness were correlated negatively. This provides further explanation as to why calcium reduction improved softening of Nabulsi cheeses.

Table 3. Hardness (N), cohesiveness, adhesiveness (J/m³), and gumminess (N) of Nabulsi cheeses stored in 4 levels of NaCl and KCl during storage for 5 mo at room temperature¹

Storage (mo)	Salt treatment ²	Hardness	Cohesiveness	Adhesiveness	Gumminess
0	A	178.0 ± 35.1 ^a	2.66 ± 0.54 ^b	0.03 ± 0.01 ^b	505.3 ± 67.8 ^a
	B	188.9 ± 7.2 ^a	3.86 ± 0.37 ^a	0.12 ± 0.02 ^a	722.9 ± 47.3 ^a
	C	200.6 ± 12.1 ^a	3.14 ± 0.08 ^{ab}	0.03 ± 0.02 ^b	631.5 ± 50.4 ^a
	D	176.5 ± 22.4 ^a	3.26 ± 0.14 ^{ab}	0.04 ± 0.03 ^{ab}	569.3 ± 54.0 ^a
1	A	209.3 ± 43.3 ^a	5.70 ± 0.35 ^a	0.09 ± 0.02 ^b	1,207.7 ± 99.9 ^a
	B	143.1 ± 4.3 ^{ab}	4.74 ± 0.05 ^b	0.17 ± 0.04 ^{ab}	678.0 ± 20.7 ^b
	C	140.0 ± 7.2 ^{ab}	4.90 ± 0.21 ^b	0.15 ± 0.03 ^{ab}	687.7 ± 57.4 ^b
	D	137.2 ± 0.06 ^b	4.57 ± 0.09 ^b	0.20 ± 0.01 ^a	627.2 ± 12.0 ^b
2	A	137.7 ± 12.9 ^a	5.08 ± 0.23 ^a	0.10 ± 0.05 ^b	703.9 ± 91.5 ^a
	B	109.8 ± 3.0 ^{bc}	5.01 ± 0.43 ^a	0.15 ± 0.04 ^{ab}	552.3 ± 59.4 ^{ab}
	C	125.1 ± 8.2 ^{ab}	4.38 ± 0.19 ^a	0.06 ± 0.03 ^b	550.7 ± 56.1 ^{ab}
	D	88.6 ± 3.8 ^c	4.46 ± 0.09 ^a	0.27 ± 0.04 ^a	395.0 ± 18.2 ^b
3	A	103.9 ± 6.7 ^b	6.65 ± 0.06 ^a	0.06 ± 0.13 ^a	691.9 ± 51.3 ^a
	B	131.7 ± 8.6 ^a	5.15 ± 0.22 ^b	0.03 ± 0.04 ^a	679.9 ± 63.2 ^a
	C	124.1 ± 1.8 ^{ab}	5.13 ± 0.31 ^b	0.13 ± 0.05 ^a	636.3 ± 42.9 ^a
	D	106.0 ± 11.9 ^{ab}	5.28 ± 0.44 ^b	0.31 ± 0.18 ^a	549.3 ± 22.6 ^a
4	A	118.4 ± 8.0 ^a	5.76 ± 0.11 ^a	0.03 ± 0.03 ^a	683.7 ± 60.3 ^a
	B	111.7 ± 7.2 ^a	4.33 ± 0.18 ^c	0.17 ± 0.08 ^a	483.9 ± 7.0 ^b
	C	112.3 ± 7.3 ^a	5.07 ± 0.34 ^b	0.11 ± 0.06 ^a	568.8 ± 56.1 ^{ab}
	D	101.7 ± 6.1 ^a	5.20 ± 0.11 ^{ab}	0.09 ± 0.05 ^a	528.7 ± 37.8 ^{ab}
5	A	133.0 ± 28.6 ^a	6.77 ± 0.11 ^a	0.08 ± 0.08 ^a	906.0 ± 25.8 ^a
	B	128.4 ± 7.6 ^a	5.30 ± 0.34 ^b	0.13 ± 0.08 ^a	684.2 ± 74.6 ^{ab}
	C	112.6 ± 3.5 ^a	5.24 ± 0.09 ^b	0.12 ± 0.06 ^a	591.0 ± 27.5 ^{ab}
	D	89.6 ± 2.6 ^a	5.25 ± 0.30 ^b	0.21 ± 0.09 ^a	472.0 ± 38.6 ^b

^{a-c}Means in each column and at the same storage time with same letter did not differ significantly ($P > 0.05$).

¹Mean values ± SE of 3 trials.

²Salt treatment: A = NaCl only (control); B = 3NaCl:1KCl (wt/wt); C = 1NaCl:1KCl (wt/wt); D = 1NaCl:3KCl (wt/wt).

Na, K, Ca, and P

The concentrations (mg/100 g of cheese) of Na, K, Ca, and P in the 4 experimental cheeses during storage are presented in Figures 2, 3, 4, and 5, respectively. The content of Na varied ($P < 0.05$) with brine solution and

storage time (Figure 2). The difference in Na content in cheeses kept in 4 brine solutions was significant ($P < 0.05$): Na contents in Nabulsi cheeses ranked in the order A > B > C > D, which was in line with the amount of Na in cheeses. Analysis of variance showed that Na contents increased ($P < 0.05$) in experimental

Figure 2. Sodium contents of Nabulsi cheeses kept with 4 levels of NaCl and KCl: A = NaCl only (control); B = 3NaCl:1KCl (wt/wt); C = 1NaCl:1KCl (wt/wt); D = 1NaCl:3KCl (wt/wt), during storage for 5 mo at room temperature.

Figure 3. Potassium contents of Nabulsi cheeses kept with 4 levels of NaCl and KCl: A = NaCl only (control); B = 3NaCl:1KCl (wt/wt); C = 1NaCl:1KCl (wt/wt); D = 1NaCl:3KCl (wt/wt), during storage for 5 mo at room temperature.

cheeses during storage for all salt treatments except for D. This may be due to Na migration from high concentration (brine solution) to lower concentration in cheese loaves (Geurts et al., 1974). The Na content in Nabulsi cheeses stored in brine solution D increased ($P > 0.05$) during storage, which may be due to the high penetra-

tion factor of K compared with Na and the lower Na than K concentration in brine solution D. Zorrilla and Rubiolo (1994) reported that KCl concentrations in the inner rings of each slice of Fynbo cheese were greater than NaCl concentrations. These findings agree with those of other researchers (Katsiari et al., 1997, 1998;

Figure 4. Calcium contents of Nabulsi cheeses kept with 4 levels of NaCl and KCl: A = NaCl only (control); B = 3NaCl:1KCl (wt/wt); C = 1NaCl:1KCl (wt/wt); D = 1NaCl:3KCl (wt/wt), during storage for 5 mo at room temperature.

Figure 5. Phosphorus contents of Nabulsi cheeses kept with 4 levels of NaCl and KCl: A = NaCl only (control); B = 3NaCl:1KCl (wt/wt); C = 1NaCl:1KCl (wt/wt); D = 1NaCl:3KCl (wt/wt), during storage for 5 mo at room temperature.

Ayyash and Shah, 2010a), who reported that Na contents increased during storage in Feta, Kefalograviera, and Halloumi cheeses.

The content of K varied ($P < 0.05$) depending on the brine solution and storage time (Figure 3). The

differences in K content among experimental cheeses kept in 4 brine solutions were significant ($P < 0.05$): K contents in Nabulsi cheeses ranked in the order $D > C > B > A$, according to brine solutions. Analysis of variance showed that K contents increased ($P <$

Figure 6. Environmental scanning electron micrograph of Nabulsi cheeses kept with 4 levels of NaCl and KCl: A = NaCl only (control); B = 3NaCl:1KCl (wt/wt); C = 1NaCl:1KCl (wt/wt); D = 1NaCl:3KCl (wt/wt), at 0 mo (panel A) and 5 mo (panel B). The individual micrographs correspond to treatments A, B, C, and D (from left to right).

0.05) in experimental cheeses during storage for all salt treatments except for A. The K contents of Nabulsi cheeses kept in brine solution A decreased ($P > 0.05$) during storage. This trend may be attributed to the high K content that already existed in cheeses for treatment A; hence, K migrated from cheeses to the brine to reach equilibrium between cheeses and brine solution.

Analysis of variance showed that Ca and P contents in experimental cheese decreased ($P < 0.05$) during storage within a salt treatment. This may be attributed to migration of Ca and P with moisture migration from cheese to brine solution (Ayyash and Shah, 2010a). Pearson correlation values between Ca and P ranged from 0.96 to 0.98. No significant ($P > 0.05$) differences were observed in Ca and P contents between experimental cheeses at the same storage time. These findings are in accordance with Ayyash and Shah (2010a), who reported a similar trend for Ca in Halloumi cheese kept in 4 brine solutions.

Microstructure

The ESEM images of experimental cheeses at 0 and 5 mo of storage are shown in Figure 6 (panels A and B, respectively). Micrographs show compact, rough, and closed structures with small voids in all experimental cheeses. Microstructure of Nabulsi cheeses kept in various NaCl and KCl mixtures did not appear to differ compared with control cheeses. After 5 mo of storage, all experimental cheeses showed smooth texture compared with cheeses at 0 mo. This may be related to a reduction in Ca content during storage, resulting in a smooth and more homogeneous cheese microstructure (Joshi et al., 2004) and an increase in proteolytic activity during storage, which in turn increased intermediate products that could hold water and close voids in protein network.

CONCLUSIONS

Storage of Nabulsi cheeses in brine solutions partially substituted with KCl at 3NaCl:1KCl, 1NaCl:1KCl, or 1NaCl:3KCl did not significantly affect chemical composition or texture profile characteristics. Proteolytic activity of Nabulsi cheeses kept in brine solutions with a higher amount of KCl was greater compared with that in control cheeses. Penetration of KCl was greater than that of NaCl in the brine solution. Storage time significantly affected the moisture, ash, protein, proteolysis (WSN and TCA-SN), Ca, P, total viable count, hardness, and gumminess of Nabulsi cheeses within a salt treatment.

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