

## The effects of salt reduction on characteristics of hard type cheese made using high proteolytic starter culture

<sup>1</sup>Sheibani, A., <sup>2</sup>Ayyash M. M., <sup>3</sup>Shah, N. P. and <sup>1\*</sup>Mishra, V. K.

<sup>1</sup>Advanced Food Systems Research Unit, College of Health and Biomedicine, Victoria University, Werribee Campus, PO Box 14428, Melbourne, Victoria 8001, Australia

<sup>2</sup>Department of Food Science, College of Food and Agriculture, United Arab Emirates University, Al Ain - United Arab Emirates

<sup>3</sup>Food and Nutritional Science, School of Biological Sciences, the University of Hong Kong, Pokfulam Road, Hong Kong

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### Abstract

The effects of salt reduction on characteristics of a hard cheese prepared using high proteolytic starter cultures were investigated. Cheeses were made with 1, 1.5, 2 and 2.5% salt and analysed for composition, starter bacteria count, proteolysis, ACE-inhibitory activity, texture profile, and microstructure before and after storage for 8 weeks. Salt reduction significantly increased proteolysis ( $p < 0.05$ ) and ACE inhibition, decreased hardness and increased compactness of cheese matrix. No differences in the sensory attributes were noted after storage for 8 weeks in cheeses made with 1.5% and 2% salt compared to the control (2.5% salt).

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### Introduction

Salt has been used in food preparation since time immemorial and added in modern food manufacturing processes for its sensory and preservation properties. In cheese, salt contributes to flavour (Guinee, 2004a), accelerates whey removal from the milled curd and controls the growth of undesirable bacteria (Emmons and Modler, 2010). Excessive intake of salt has been associated with high blood pressure, kidney stones and osteoporosis (Turk *et al.*, 2009). Therefore, health authorities in the U.S.A have recommended a limitation on dietary intake of sodium to about 2.5-3 g per day (McCarron *et al.*, 2009). The World Health Organization (WHO) also recommended to reduce average salt consumption for adults to  $< 5$  g per day (Flock and Kris-Etherton, 2011). The daily intake of salt in the Australian diet is over 3-5 g per day (National Health and Medical Research Council, 2003) and must be reduced to match the recommendations.

Cheeses contribute about 4% of the daily sodium intake in United Kingdom (Ash and Wilbey, 2010), 9.2% in France (Meneton *et al.*, 2009), and 5% in Australia (National Health and Medical Research Council, 2003). Reduction of salt in cheese will assist in meeting the recommended daily dietary intake of sodium. This is particularly significant as cheese is one of the popular dairy product and its consumption

is likely to increase (Fox and McSweeney, 2004). It has been estimated that the dietary sodium intake would decrease to approximately 20% (He and MacGregor, 2010) by consuming cheeses with medium salt content (2% w/w). Reduction or substitution of salt can induce subtle and significant changes in quality of cheese (Guinee, 2004b). Lack of flavour, loss of desired texture and excessive growth of microorganism in cheese are known quality defects associated with salt reduction (Guinee and Fox, 2004) by influencing the biochemistry of the ripening processes. One of the approaches used is to augment starter culture with adjunct cultures having significant proteolytic activities to compensate for the lost texture and flavor (Børsting *et al.*, 2012, Sato *et al.*, 2012).

Replacement of sodium chloride (NaCl) with other salts, such as KCl and  $MgCl_2$ , also leads to noticeable bitterness and metallic taste (Sheibani *et al.*, 2013). McMahan *et al.* (2009) reported that moisture content of feta cheeses decreased when brine concentration increased at 3°C. The hardness of fat-reduced Cheddar cheese showed a decrease due to reduction of salt in moisture (SM) and increase in moisture (Mistry and Kasperson, 1998). In other studies, partial substitution of NaCl with KCl did not show significant effect on proteolysis of feta cheese (Merćep *et al.*, 2010) and Minas cheese, a semi-soft white Brazilian cheese (Gomes *et al.*, 2011). Also, no

\*Corresponding author.

Email: [vijay.mishra@vu.edu.au](mailto:vijay.mishra@vu.edu.au)

Tel: +61 3 99198130

significant difference in the texture characteristics of Halloumi cheese was found upon partial substitution of salt with KCl (Ayyash *et al.*, 2011).

The objective of this study was to investigate the effect of salt reduction on chemical composition, starter culture growth, proteolysis, anti-hypertensive properties, microstructure, and textural profile of cheese made with a high proteolytic starter cultures (*Streptococcus thermophilus* and *Lactobacillus helveticus*) during storage at 4.5°C for 8 weeks.

## Materials and Methods

### Cheese making

One hundred litre of full-cream cow milk (3.6% protein, 4.0% fat and 4.7% lactose) was standardized with skim milk to give a casein/fat ratio of 0.70 and then pasteurized at 65°C for 30 min, cooled to 32°C and divided into four 25 L batches. Each batch was transferred into a 25 L cheese vat (Unipulse Pty. Ltd, Victoria, Australia) and inoculated with a commercial freeze-dried cheese starter culture (2.5% (w/v) based on milk; TCC-20 Chr. Hansen, Bayswater, Victoria, Australia) consisting of *Streptococcus thermophilus* and *Lactobacillus helveticus* following the supplier's instructions (Chr. Hansen). After 35 min of agitation, and when the pH of the milk had dropped by about 0.3 units, 5 mL of double-strength rennet (CHY-Max, Chr. Hansen), diluted in 100 mL of distilled water, was added to the milk, followed by mixing for 1 min. The milk coagulated in 35 min and the curd was cut into 1 cm<sup>3</sup> cubes using cheese wire knives, was allowed to stand for 10 min followed by stirring and gradual heating up to 38°C within approximately 50 min. The whey was drained and curd was milled at pH 5.2 - 5.3.

The milled curds were salted at 4 different levels of NaCl [(2.5% w/w as Control (C), 2% w/w for Treatment 1 (T1), 1.5% w/w for Treatment 2 (T2), and 1% w/w for Treatment 3 (T3)] and were mellowed for 10 min. The salted curds were hooped in 2.5 kg capacity molds and pressed at a pressure of 0.0246 kg/cm<sup>2</sup> overnight. Pressed curd was cut into 250 g blocks and vacuum packaged in plastic bags and stored at 4.5°C for 8 weeks. Samples were collected every 2 weeks of storage for analyses from packaged cheese corresponding to each treatment. Whole experiment was repeated independently three times.

### Chemical composition

Moisture was determined by the oven-drying method using acid-washed sand (Sigma, St. Louis, Mo., U.S.A.) at 102°C, fat by the Babcock method, protein by the Kjeldahl method, and ash by the muffle

furnace method according to Association of Official Analytical Chemists methods (AOAC International, 1995). For pH measurement, 20 g of grated cheese was macerated with 20 mL distilled water, and the pH of the resulting slurry was measured by a digital pH meter (MeterLab, Pacific Laboratory Products, Blackburn, Victoria, Australia) after calibration. All analyses were carried out in duplicate.

### Starter lactic acid bacteria (SLAB) enumeration

*Streptococcus thermophilus* and *Lactobacillus helveticus* in cheese were enumerated by the pour-plating method as described by Tharmaraj and Shah (2003). Briefly, 11 g grated cheese and 99 mL sterile distilled water were blended for 2 min in a stomacher-400 laboratory blender (Seward Medical, London, U.K.). Serial dilutions were made in sterilized solutions of 0.1% peptone and water (Sigma). *L. helveticus* and *S. thermophilus* were grown on de Man, Rogosa, and Sharpe (MRS) agar and M17 agar (Merck Pty. Ltd., Victoria, Australia), respectively. Inoculated plates of MRS agar were incubated anaerobically (using anaerobic jars) and M17 agar aerobically at 37°C for 48 h.

### Proteolysis assessment

The water-soluble extract (WSE) of each cheese sample was prepared according to Kuchroo and Fox (1982). Briefly, a mixture of cheese and distilled water (1:2) was made and kept in a 40°C water bath for 60 min following by centrifugation at 4000× g for 30 min. The resultant slurry was filtered through a 0.45-µm filter (Millipore Corp., Bedford, MA). The total nitrogen in the extract was estimated by the Kjeldahl method (AOAC International, 1995). 12% Trichloroacetic acid-soluble nitrogen (TCA-SN) (Sigma) was estimated in 9 mL filtrate obtained after 5 mL of WSE was mixed with 5 mL of 24% TCA and left overnight followed by centrifugation at 4000× g for 20 min. 5% Phosphotungstic acid-soluble nitrogen (PTA-SN) was measured in 9 mL filtrate obtained after 5 mL of WSE was mixed with 5 mL of 10% PTA (Sigma) and left overnight followed by centrifugation at 4000× g for 20 min.

### Measurement of total free amino acids

Concentrations of total free amino acids (TFAA) in WSE of experimental cheeses were measured by using the Cd-ninhydrin method according to Folkertsma and Fox (1992).

### Angiotensin-converting enzyme (ACE) inhibitor

### activity

The ACE inhibitory activity of WSE of the experimental cheeses was measured according to Cushman and Cheung (1971) with minor modifications as reported by Ayyash and Shah (2011). The percentage inhibition of enzyme activity was calculated as follows:

$$\% \text{ ACE-Inhibition} = [\text{HA (control)} - \text{HA (sample)}] / \text{HA (control)} \times 100$$

### Determination of sodium content

A multitype inductively coupled plasma atomic emission spectrometer (ICPE-9000; Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Rydalmere, NSW, Australia) was employed for the determination of sodium content in cheeses according to Ayyash and Shah (2010).

### Texture profile analysis

The texture profile was obtained according to method of Bryant *et al.* (1995) with some modifications. Cheese cylinders (30mm height × 20mm diameters) were cut from the center of experimental cheese blocks. Hardness, cohesiveness, adhesiveness, and gumminess were measured using Instron universal testing machine (model 5564; Instron Ltd., London, UK) as described by Pons and Fiszman (1996). The samples were compressed to 30% of their original height using a 500-N load cell with a flat plunger and the crosshead movement was adjusted to 30 mm per min. Double-compression was achieved and the data were collected using Merline software. Analyses were performed in triplicate.

### Cheese microstructure

The microstructure of cheeses was monitored by Environmental Scanning Electron Microscopy as described by Ayyash *et al.* (2011). Briefly, 0.5 cm<sup>3</sup> cubes of cheese were cut from the center of cheese block and were imaged by FEI quanta environmental scanning electron microscopy (ESEM; Philips Electron Optics, Eindhoven, the Netherlands) using ESEM mode. Images were taken at an accelerating voltage at 30 kV under vacuum (0.47 kPa) using 1,000 × magnification at 4°C.

### Sensory Evaluation

Sensory evaluation was conducted after the human ethic application has been approved by Victoria University human ethic committee (approval ID HRE13-005). Fourteen panellists were recruited from Victoria University staff and research students for assessing sensory attributes of experimental

cheese samples using 10 point hedonic test. The panellists were trained for their ability to detect creaminess, bitterness, saltiness, sour-acid, and vinegary tastes. Sensory evaluation was conducted for cheeses at weeks 0 and 8 of storage. Cheese samples were tempered at room temperature (20°C) for 1 h and cut into pieces and placed on white plates coded with random 3-digit numbers. The panellists evaluated 4 samples per session. Crackers and water were provided between samples to change the taste and rinse palate and taste buds. The panellists ranked the attributes using a 10-point scale as follows: creaminess 1 = absence of creamy taste and 10 = extremely creamy taste, similarly, bitterness, saltiness, salty, sourness, vinegary, and acceptability 1 = not accepted and 10 = highly accepted.

### Statistical analysis

One-way ANOVA was carried out to examine the significant difference between experimental cheeses for same storage time. Two-way ANOVA was performed to investigate the effects of salting treatment and storage time on the cheese characteristics at  $p < 0.05$ . Fisher's least significant difference was carried out to explore the significant differences between means at the same storage period. All data were statistically analyzed using SAS 9.0 software (SAS Institute Inc, 2008).

## Results and Discussion

### Chemical composition

The chemical compositions of experimental cheeses using different salt levels at day 0 (right after pressing) are presented in Table 1. Salt reduction significantly ( $p < 0.05$ ) affected the moisture and salt-in-moisture (S/M) contents of the experimental cheeses. The moisture content of salt reduced treatments (T1, T2, and T3) was significantly ( $p < 0.05$ ) higher, while their S/M contents were lower ( $p < 0.05$ ) than control (C). The order of moisture contents was as follows: T3 > T2 > T1 > C, whereas S/M had the opposite order. These results are in agreement with those of Schroeder *et al.* (1988) who also reduced the salt content in Cheddar cheese and showed that moisture content of high-salted Cheddar cheese was lower than other treatments. Sodium content expectedly differed significantly ( $p < 0.05$ ) between experimental cheeses (Table 1). Ash and sodium contents increased in the same proportion as the treatment salt concentration used. However, there was no change in the fat and protein contents, and the pH values.

*Proteolysis and starter lactic acid bacteria (SLAB)*

Table 1. Chemical composition, sodium content and pH of experimental cheeses made with 4 levels of NaCl at day 0 (mean± SE of 3 trials)

Salt treatment	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	pH	Sodium (mg/100g)	S/M (%)
C	33.89±1.12 <sup>c</sup>	23.87±0.43 <sup>a</sup>	35.67±0.60 <sup>a</sup>	1.97±0.98 <sup>a</sup>	4.91±0.08 <sup>a</sup>	594.24±18.4 <sup>a</sup>	4.40±0.28 <sup>a</sup>
T1	36.93±1.11 <sup>b</sup>	26.32±2.06 <sup>a</sup>	37.17±0.44 <sup>a</sup>	1.53±0.78 <sup>b</sup>	4.97±0.02 <sup>a</sup>	492.37±52.9 <sup>ab</sup>	3.07±0.52 <sup>b</sup>
T2	38.45±0.23 <sup>ab</sup>	23.21±1.19 <sup>a</sup>	36.33±1.45 <sup>a</sup>	1.37±0.67 <sup>c</sup>	4.98±0.03 <sup>a</sup>	386.76±11.1 <sup>cb</sup>	2.52±0.08 <sup>bc</sup>
T3	39.59±0.35 <sup>a</sup>	24.61±0.97 <sup>a</sup>	35.83±1.01 <sup>a</sup>	1.07±0.54 <sup>d</sup>	5.01±0.01 <sup>a</sup>	287.81±37.0 <sup>c</sup>	1.82±0.25 <sup>c</sup>

<sup>a-c</sup> Means in each column with different letters are significantly different ( $p < 0.05$ ) at same storage period

Table 2. Proteolysis parameters, ACE-inhibitory and microbial growth of experimental cheeses made with 4 levels of NaCl during storage for 8 weeks at 4.5°C (mean± SE of 3 trials)

Storage (week)	Salt treatment	WSN	TCA-SN	PTA-SN	TFAA	ACE-inhibitory	<i>L. helveticus</i>	<i>S. thermophilus</i>
0	C	5.16±0.75 <sup>a</sup>	3.03±1.22 <sup>a</sup>	2.32±0.15 <sup>a</sup>	0.86±0.02 <sup>a</sup>	13.37±2.27 <sup>a</sup>	8.72±0.01 <sup>b</sup>	8.69±0.01 <sup>ab</sup>
	T1	5.23±1.48 <sup>a</sup>	2.35±0.40 <sup>a</sup>	2.44±0.69 <sup>a</sup>	0.84±0.01 <sup>a</sup>	12.28±1.54 <sup>a</sup>	8.67±0.04 <sup>b</sup>	8.61±0.17 <sup>b</sup>
	T2	5.75±1.49 <sup>a</sup>	3.77±0.48 <sup>a</sup>	2.33±0.04 <sup>a</sup>	0.94±0.01 <sup>b</sup>	14.82±1.28 <sup>a</sup>	8.71±0.01 <sup>b</sup>	8.66±0.02 <sup>b</sup>
	T3	5.86±1.34 <sup>a</sup>	3.43±0.96 <sup>a</sup>	2.42±0.09 <sup>a</sup>	0.99±0.03 <sup>b</sup>	14.93±0.90 <sup>a</sup>	9.21±0.02 <sup>a</sup>	8.97±0.02 <sup>a</sup>
2	C	7.45±0.53 <sup>a</sup>	3.05±0.03 <sup>a</sup>	2.33±0.40 <sup>b</sup>	0.81±0.02 <sup>a</sup>	14.56±0.78 <sup>a</sup>	9.38±0.07 <sup>b</sup>	9.58±0.04 <sup>ab</sup>
	T1	7.87±0.26 <sup>a</sup>	3.19±0.10 <sup>a</sup>	2.32±0.35 <sup>b</sup>	0.81±0.02 <sup>a</sup>	15.21±1.53 <sup>a</sup>	9.38±0.18 <sup>b</sup>	9.73±0.17 <sup>ab</sup>
	T2	7.58±0.11 <sup>a</sup>	3.84±0.57 <sup>a</sup>	2.72±0.22 <sup>a</sup>	0.87±0.04 <sup>a</sup>	14.94±2.14 <sup>a</sup>	8.54±0.03 <sup>b</sup>	9.43±0.01 <sup>b</sup>
	T3	7.63±0.37 <sup>a</sup>	3.29±0.10 <sup>a</sup>	2.80±0.36 <sup>a</sup>	0.99±0.03 <sup>b</sup>	15.88±1.91 <sup>a</sup>	9.63±0.29 <sup>a</sup>	9.75±0.05 <sup>a</sup>
4	C	11.90±0.93 <sup>a</sup>	3.98±0.19 <sup>a</sup>	2.60±0.12 <sup>b</sup>	0.87±0.01 <sup>a</sup>	17.92±0.47 <sup>a</sup>	9.10±0.17 <sup>b</sup>	9.86±0.11 <sup>b</sup>
	T1	10.77±0.60 <sup>a</sup>	3.99±0.29 <sup>a</sup>	2.88±0.22 <sup>b</sup>	0.89±0.01 <sup>a</sup>	16.24±0.96 <sup>a</sup>	9.32±0.01 <sup>b</sup>	9.36±0.02 <sup>b</sup>
	T2	11.29±0.13 <sup>a</sup>	4.48±0.17 <sup>a</sup>	3.92±0.23 <sup>a</sup>	0.90±0.00 <sup>a</sup>	15.36±1.48 <sup>a</sup>	9.36±0.01 <sup>a</sup>	9.34±0.01 <sup>b</sup>
	T3	10.45±0.36 <sup>a</sup>	4.53±0.11 <sup>a</sup>	2.97±0.09 <sup>ab</sup>	0.99±0.00 <sup>b</sup>	17.54±0.40 <sup>a</sup>	9.36±0.01 <sup>a</sup>	9.36±0.01 <sup>a</sup>
6	C	9.32±0.19 <sup>a</sup>	4.28±0.11 <sup>a</sup>	2.88±0.13 <sup>b</sup>	0.89±0.06 <sup>a</sup>	15.26±0.54 <sup>a</sup>	9.34±0.05 <sup>b</sup>	9.35±0.02 <sup>b</sup>
	T1	9.52±0.48 <sup>a</sup>	4.52±0.33 <sup>a</sup>	2.33±0.39 <sup>b</sup>	0.91±0.01 <sup>a</sup>	15.11±0.89 <sup>a</sup>	9.37±0.01 <sup>b</sup>	9.27±0.04 <sup>b</sup>
	T2	9.92±0.48 <sup>a</sup>	5.34±0.08 <sup>a</sup>	3.44±0.53 <sup>a</sup>	0.96±0.02 <sup>b</sup>	17.77±0.08 <sup>a</sup>	9.32±0.04 <sup>b</sup>	9.35±0.01 <sup>b</sup>
	T3	9.87±0.27 <sup>a</sup>	5.32±0.55 <sup>a</sup>	3.71±0.15 <sup>a</sup>	1.09±0.06 <sup>b</sup>	18.26±0.55 <sup>a</sup>	9.65±0.01 <sup>a</sup>	9.79±0.04 <sup>a</sup>
8	C	12.09±0.65 <sup>a</sup>	4.66±0.19 <sup>a</sup>	2.83±0.62 <sup>ab</sup>	0.91±0.03 <sup>a</sup>	16.95±2.02 <sup>a</sup>	9.03±0.09 <sup>b</sup>	9.09±0.06 <sup>b</sup>
	T1	10.92±0.69 <sup>a</sup>	4.55±0.27 <sup>a</sup>	2.38±0.27 <sup>b</sup>	0.94±0.08 <sup>a</sup>	17.71±1.10 <sup>a</sup>	9.01±0.08 <sup>b</sup>	9.09±0.08 <sup>b</sup>
	T2	11.08±0.15 <sup>a</sup>	4.98±0.34 <sup>a</sup>	3.08±0.16 <sup>a</sup>	0.99±0.06 <sup>b</sup>	18.52±0.09 <sup>a</sup>	9.00±0.06 <sup>b</sup>	9.21±0.01 <sup>ab</sup>
	T3	12.31±0.49 <sup>a</sup>	4.30±0.21 <sup>a</sup>	3.77±0.23 <sup>a</sup>	1.13±0.04 <sup>b</sup>	19.58±4.92 <sup>a</sup>	9.20±0.05 <sup>a</sup>	9.26±0.01 <sup>a</sup>

<sup>a-c</sup> Means in each column with different letters are significantly different ( $p < 0.05$ ) at same storage period

### enumeration

The influence of salt reduction on the growth of starter culture (*L. helveticus* and *S. thermophilus*) and proteolysis parameters of experimental cheeses stored at 4.5°C for 8 weeks is presented in Table 2. Salt reduction had a significant effect ( $p < 0.05$ ) on starter culture cell growth. At the same storage period, *L. helveticus* and *S. thermophilus* growths in treatment 3 (T3) containing the lowest salt content were significantly ( $p < 0.05$ ) higher than in the other cheeses. This is due to higher water activity, which is a key requirements for microbiological activity (Guinee and Fox, 2004), corresponding to low salt content in T3 cheeses.

Proteolysis during ripening stage of cheese is characterized by primary indicated by WSN, intermediate by TCA-SN and advanced by PTA-SN (McSweeney and Fox, 1997). Significant ( $p < 0.05$ ) increase in WSN and TCA-SN was observed between week 0 and 8 in all experimental cheeses within the same salt treatment (Table 2). These

results are in agreement with those of Aly (1995) and Schroeder *et al.* (1988) who reported an increase in proteolysis of low-salt Feta and Cheddar cheeses, respectively, during storage. Further, the increase in proteolysis during storage may be attributed to the residual rennet and starter culture activities in cheeses (Sousa *et al.*, 2001). At the same storage time, Table 2 shows that the differences in WSN and TCA-SN contents were observed between experimental cheeses were insignificant ( $p > 0.05$ ), while the differences in PTA-SN was significant ( $p < 0.05$ ). PTA-SN of experimental cheeses salted at 2.5% (C) and 2.0% (T1) were significantly ( $p < 0.05$ ) lower than other cheeses (T2 and T3) at week 2, 4, 6 and 8 of storage. This lower PTA-SN content in C and T1 treatments shows inhibition of the proteolytic activities of enzymes produced by the starter cultures as previously been reported by (Guinee, 2004a).

Table 3. Texture profile of experimental cheeses made with 4 levels of NaCl during storage for 8 weeks at 4.5°C (mean± SE of 3 trials)

Storage (week)	Salt treatment	Hardness	Cohesiveness	Adhesiveness	Gumminess
0	C	13.17±0.47 <sup>b</sup>	0.760±0.01 <sup>a</sup>	0.120±0.03 <sup>b</sup>	10.00±0.25 <sup>a</sup>
	T1	18.11±1.39 <sup>a</sup>	0.690±0.03 <sup>ab</sup>	0.210±0.05 <sup>a</sup>	12.60±1.37 <sup>a</sup>
	T2	12.54±0.35 <sup>c</sup>	0.700±0.01 <sup>ab</sup>	0.140±0.02 <sup>b</sup>	8.77±0.21 <sup>b</sup>
	T3	12.27±1.79 <sup>c</sup>	0.640±0.04 <sup>b</sup>	0.230±0.01 <sup>a</sup>	7.85±1.57 <sup>b</sup>
2	C	8.32±0.74 <sup>a</sup>	0.720±0.00 <sup>b</sup>	0.520±0.10 <sup>b</sup>	6.00±0.56 <sup>a</sup>
	T1	6.45±0.36 <sup>b</sup>	0.790±0.02 <sup>a</sup>	0.980±0.19 <sup>a</sup>	5.10±0.35 <sup>a</sup>
	T2	4.50±0.67 <sup>a</sup>	0.660±0.03 <sup>c</sup>	0.580±0.07 <sup>b</sup>	2.99±0.51 <sup>b</sup>
	T3	4.25±0.21 <sup>c</sup>	0.710±0.01 <sup>bc</sup>	0.970±0.08 <sup>a</sup>	3.00±0.17 <sup>b</sup>
4	C	7.26±0.14 <sup>a</sup>	0.700±0.03 <sup>b</sup>	0.570±0.02 <sup>b</sup>	5.17±0.19 <sup>a</sup>
	T1	7.19±0.36 <sup>a</sup>	0.720±0.10 <sup>b</sup>	0.640±0.03 <sup>a</sup>	5.70±0.60 <sup>a</sup>
	T2	6.84±0.45 <sup>a</sup>	0.730±0.16 <sup>a</sup>	0.530±0.08 <sup>b</sup>	5.09±0.97 <sup>b</sup>
	T3	5.10±0.47 <sup>b</sup>	0.630±0.16 <sup>b</sup>	0.650±0.22 <sup>a</sup>	3.08±0.60 <sup>c</sup>
6	C	10.14±0.76 <sup>ab</sup>	0.600±0.07 <sup>b</sup>	0.550±0.04 <sup>b</sup>	6.15±1.05 <sup>b</sup>
	T1	11.78±0.72 <sup>a</sup>	0.740±0.01 <sup>a</sup>	0.700±0.03 <sup>a</sup>	8.68±0.47 <sup>a</sup>
	T2	9.19±0.71 <sup>cb</sup>	0.710±0.02 <sup>ab</sup>	0.550±0.07 <sup>b</sup>	6.02±0.39 <sup>bc</sup>
	T3	7.37±0.45 <sup>c</sup>	0.700±0.01 <sup>ab</sup>	0.620±0.03 <sup>a</sup>	5.14±0.24 <sup>c</sup>
8	C	11.06±0.85 <sup>b</sup>	0.750±0.03 <sup>b</sup>	0.580±0.19 <sup>b</sup>	7.06±0.68 <sup>a</sup>
	T1	13.75±1.22 <sup>a</sup>	0.760±0.02 <sup>a</sup>	0.760±0.12 <sup>a</sup>	10.36±0.78 <sup>a</sup>
	T2	9.94±0.62 <sup>bc</sup>	0.530±0.02 <sup>a</sup>	0.570±0.09 <sup>b</sup>	6.20±0.37 <sup>b</sup>
	T3	8.31±0.34 <sup>c</sup>	0.730±0.02 <sup>a</sup>	0.670±0.20 <sup>a</sup>	6.07±0.36 <sup>b</sup>

<sup>a-c</sup> Means in each column with different letters are significantly different ( $p < 0.05$ ) at same storage period

Table 4. Sensory attributes of experimental cheeses (week 0 and 8) made with 4 levels of NaCl and ripened at 4.5°C (mean± SE of 3 trials)

Storage (week)	Salt treatment	Creaminess	Sour-acid	Saltiness	Bitterness	Vinegary	Acceptability
0	C	5.87±0.58 <sup>a</sup>	3.40±0.63 <sup>a</sup>	6.26±0.55 <sup>a</sup>	1.94±0.61 <sup>a</sup>	3.16±0.63 <sup>a</sup>	6.44±0.49 <sup>a</sup>
	T1	6.23±0.40 <sup>a</sup>	3.66±0.68 <sup>a</sup>	6.22±0.59 <sup>a</sup>	2.23±0.60 <sup>a</sup>	2.73±0.59 <sup>a</sup>	5.54±0.74 <sup>a</sup>
	T2	6.05±0.41 <sup>a</sup>	3.59±0.60 <sup>a</sup>	5.83±0.52 <sup>b</sup>	2.01±0.55 <sup>a</sup>	2.87±0.57 <sup>a</sup>	6.23±0.41 <sup>a</sup>
	T3	5.37±0.56 <sup>a</sup>	3.87±0.58 <sup>a</sup>	5.70±0.59 <sup>b</sup>	1.80±0.55 <sup>a</sup>	2.73±0.70 <sup>a</sup>	6.30±0.59 <sup>a</sup>
8	C	6.36±0.55 <sup>a</sup>	4.75±0.55 <sup>a</sup>	6.53±0.51 <sup>a</sup>	5.32±0.75 <sup>a</sup>	3.93±0.61 <sup>b</sup>	6.21±0.61 <sup>a</sup>
	T1	5.93±0.50 <sup>a</sup>	5.36±0.59 <sup>a</sup>	5.60±0.49 <sup>a</sup>	5.29±0.83 <sup>a</sup>	3.93±0.65 <sup>b</sup>	6.03±0.64 <sup>a</sup>
	T2	5.93±0.60 <sup>a</sup>	5.50±0.64 <sup>a</sup>	5.52±0.66 <sup>ab</sup>	5.93±0.80 <sup>a</sup>	4.86±0.72 <sup>a</sup>	5.87±0.74 <sup>a</sup>
	T3	5.93±0.54 <sup>a</sup>	5.43±0.56 <sup>a</sup>	4.84±0.50 <sup>b</sup>	6.25±0.66 <sup>a</sup>	4.39±0.63 <sup>a</sup>	4.39±0.58 <sup>b</sup>

<sup>a-c</sup> Means in each column with different letters are significantly different ( $p < 0.05$ ) at same storage period

#### Total free amino acids

Development of total free amino acids (TFAA) of the experimental cheeses during storage is shown in Table 2 as absorbance. TFAA is an index of the proteolytic enzymes activity produced by SLAB, mainly those that hydrolyze small peptides to produce free amino acids (McSweeney and Sousa, 2000, Upadhyay *et al.*, 2004). The TFAA in cheeses salted with 1.5% and 1% NaCl (T2 and T3) were significantly ( $p < 0.05$ ) higher than T1 and control at the same storage period. In general, at higher the salt contents in cheese, the inhibition of proteolysis by salt led to lower amounts of total free amino acids. This may be due to the effect of higher salt content which in turn reduces the water activity and consequently the microbial growth and their proteolytic activity (Guinee and Fox, 2004). The total free amino acids expectedly increased ( $p < 0.05$ ) by the storage irrespective of the salt content in cheese.

#### ACE-inhibitory activity in WSE

ACE-inhibitory activity of experimental cheeses salted at 4 different levels and stored at 4.5°C for 8 weeks are presented in Table 2. Experimental cheeses containing higher salt levels (C and T1) had lower ACE-inhibitory activities compared with T2 and T3 at the end of storage (weeks 6 and 8). Following the proteolysis pattern as describe before, ACE-inhibitory activities increased significantly ( $p < 0.05$ ) with prolonged storage. ACE inhibitory activity was the highest at 8<sup>th</sup> week of storage for a treatment containing the least amount of salt due to residual activity of proteolytic enzymes. The increase in ACE-inhibitory activities during storage is in accordance with findings by Ong and Shah (2008) for probiotic Cheddar cheese.

#### Texture profile analysis

Texture analysis is very important for classification of cheese. Table 3 presents the texture

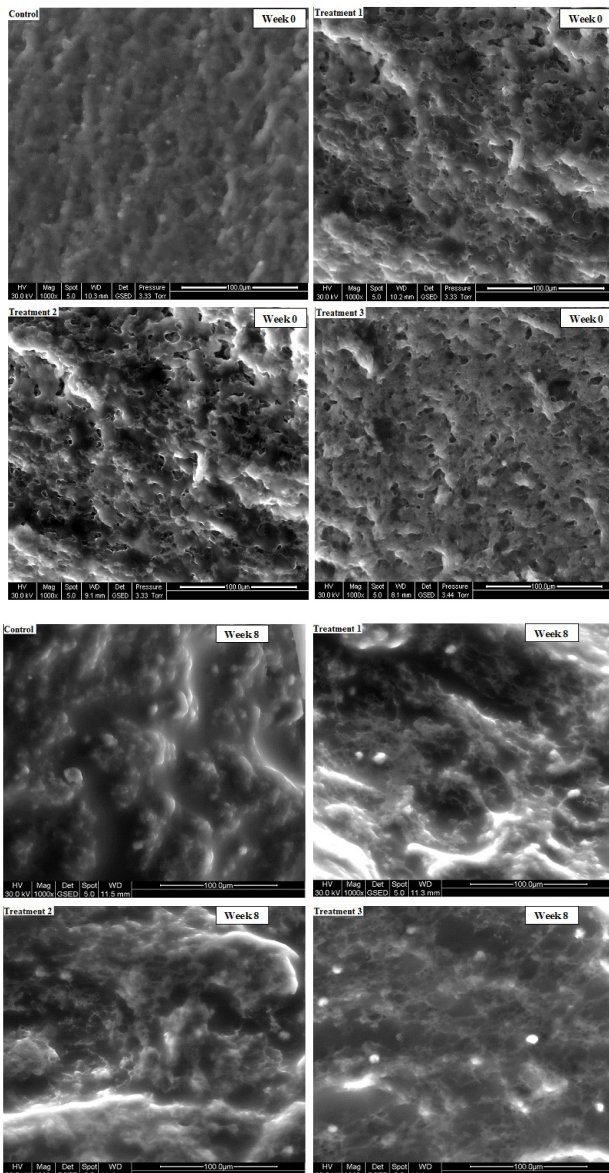


Figure 1. ESEM images of cheeses made with 4 levels of NaCl (Control=Salted at 2.5%; Treatment1= Salted at 2%; Treatment2= Salted at 1.5%; and Treatment3= Salted at 1%) during 0 and 8 week of storage at 4.5°C

profile as measured by the Instron texture analyzer for experimental cheese salted at 4 different levels and stored at 4.5°C for 8 weeks. At the same storage time, hardness and gumminess of experimental cheese salted at 1% (T3) was significantly ( $p < 0.05$ ) lower than other treatments. The low salt content increased the moisture content in T3 cheeses which contributed to decreased hardness and gumminess (Guinee, 2004a). Cohesiveness, Adhesiveness and Gumminess of experimental cheeses differed significantly ( $p < 0.05$ ). Adhesiveness showed significant difference ( $p < 0.05$ ) between experimental cheeses at the same storage time. A comparison between week 0 and 8 showed that hardness and gumminess of all experimental cheeses significantly ( $p < 0.05$ ) decreased at the same salting treatment.

These observations were due to the increase in proteolysis that occurred in cheeses during storage which softened the cheeses (Pollard *et al.*, 2003).

#### Microstructure by environmental scanning electron microscopy

The ESEM images of experimental cheeses at 0 and 8 weeks of storage are shown in Figure 1A and Figure 1B, respectively. The images show compact and closed structures with small cavities in all experimental cheeses. However, cavities decreased slightly over storage time due to the production of peptides, which blocked structural cavities in cheeses by holding water in the serum phase (Fox and McSweeney, 1996).

#### Sensory evaluation

The scores of creaminess, sour-acid, saltiness, bitterness, vinegary, and acceptability attributes of the experimental cheese samples (week 0 and 8) are presented in Table 4. At week 0, there were no significant differences between experimental cheeses in all sensory attributes except saltiness. However, significant differences between experimental cheeses were clearly observed at week 8. Cheese with 2.5% salt (control) received higher ( $p < 0.05$ ) acceptability scores than the other salt treatments. Expectedly, the lowest salted cheese samples had lower ( $p < 0.05$ ) acceptability scores compared with the other treatments. Besides saltiness, the effect of salt on proteolysis affected flavour (Upadhyay *et al.*, 2004). Vinegary attribute differed significantly ( $p < 0.05$ ) among experimental cheeses at week 8 whereas treatments T2 and T3 received higher scores compared to the control and T1. High salt content in cheeses inhibits the bacterial growth (Guinee, 2004a) which in turn reduced the production of acetic acid contributing to vinegary sensation. Sour-acid, bitterness, and vinegary taste sensations increased significantly ( $p < 0.05$ ) during storage in all experimental cheese samples. This effect may be due to production of organic acids (sour-acid), peptides (bitterness), and acetic acid (vinegary) associated with the bacteria growth in low salt containing samples.

#### Conclusion

In conclusion, salt reduction and the use of high proteolytic starter culture influenced sensory characteristics through increased proteolysis. The ACE inhibition increased with maximum inhibition (19%) corresponding to 1% salt in cheese stored for 8 weeks, cheese matrix compactness increased,

and hardness decreased upon salt reduction. It is possible to prepare cheese with 1.5 % salt without any significant changes to the sensory quality.

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