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Texture and microstructure of reduced-salt Cheddar cheese as affected by process modifications

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Abstract

The effect of change in pH at drainage (6.2, 5.9 and 5.6), rennet concentration (0.1 and 0.3) and casein to fat ratio (C/F) 0.60, 0.70 and 0.80) on texture and microstructure of salt-reduced Cheddar cheese was investigated. Cheeses with these treatments were prepared and analyzed for composition, texture profile and microstructure. At the same drainage pH, cheeses made with C/F ratio 0.6 had lower ($P < 0.05$) dry matter and protein content whereas, fat and ash % in dry matter were higher ($P < 0.05$) compared with at 0.7 and 0.8. The pH decreased significantly ($P < 0.05$) from day 0 to day 120 and then stabilized thereafter. In general, hardness of all cheeses increased from day 0 to day 60 of storage and then gradually decreased by the end of ripening (180 days). Cohesiveness showed an opposite trend to hardness. Adhesiveness significantly decreased during the storage period. Microstructure of cheese became denser, more compact and homogenous with small gaps at the end of storage (day 180) due to presence of peptides resulting from ripened cheese filling the matrix cavity.

Introduction

Texture and microstructure are two important parameters dictating the quality of food products including cheese. These parameters are highly dependent on composition, extent of proteolysis, decrease in aw through water binding by liberated carboxyl and amino groups and increase in the pH (McSweeney and Sousa, 2000). Several factors have been identified by Gunasekaran and Ak (2003) and more specifically low salt containing cheeses are normally pasty and have off flavour (Fox, 1975). For example, salt reduction from 2.5 % (salt in curd) to 1.5% has been shown to decrease hardness by 10% (Murtaza *et al*., 2014). While manufacture of cheese with low salt and fat results in less acceptable quality (Bryant *et al*., 1995, Ganesan *et al*., 2014), these components have been associated with several human health issues (Turk *et al*., 2009, Sheibani *et al*., 2013). Reduction of salt in cheese accelerates protein hydration which has a major influence on the physical properties and quality of cheese due to their influence on microstructure, rheology and texture of cheese (McSweeney and Fox, 2009).

The changes in hardness and colour of Gaziantep cheese kept in different brines concentrations (5%, 10%, 15%, 20% and 25% NaCl) followed by storage for two weeks have been monitored by Kaya (2002). Cheeses in higher concentration of brine showed higher hardness and least change in colour (whiteness) compared with the control (25% brine). Kilic and Isin (2004) examined texture of Dil cheese brined at two salt concentrations (3 and 6% NaCl) during storage for 3 months and showed that lower salt containing cheese had soft texture. This was attributed to solubilisation of proteins filling the serum phase between the protein fibres strengthening the protein network. Similarly, the texture of Cheddar cheeses made from buffalo milk with 2.5, 2.0, 1.5, 1.0, and 0.5% (w/w of the curd) salt and ripened at 6 to 8°C for 180 days showed significant decrease in hardness (from ~190 to ~ 110 N), toughness (from ~180 to ~130 kPa), and crumbliness (from ~ 3.5 to ~2.5) due to increase in proteolysis from ~19% to ~25% at the end of ripening. These samples with salt concentration <2% were less acceptable on sensory analysis (Murtaza *et al*., 2014).

Replacement of salt with replacers such as KCl and $MgCl_2$ may compensate for sensory and textural losses in cheese; however, this may trigger metallic and bitter aftertaste (Reddy and Marth, 1993, Grummer *et al*., 2012). Lefier *et al.* (1987) investigated the replacement of salt with $NaCl/MgCl_2$ mixture in Gruyere cheese. The author reported that cheeses made with $NaCl/MgCl_2$

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Table 1. Experimental design of the current study

Factors			Treatment codes
pH	C/F ratio	Rennet Concentration	
6.2	0.6	0.1	A
		0.3	B
	0.7	0.1	C
		0.3	D
	0.8	0.1	E
		0.3	F
5.9	0.6	0.1	G
		0.3	H
	0.7	0.1	I
		0.3	J
	0.8	0.1	K
		0.3	L
5.6	0.6	0.1	M
		0.3	N
	0.7	0.1	O
		0.3	P
	0.8	0.1	Q
		0.3	R

mixture showed acceptable taste with a slight bitterness and soft body. The effect of partial substitution of NaCl with KCl on Halloumi cheese brined in 4 different 18% brine solutions including only NaCl, 3NaCl: 1KCl, 1NaCl: 1KCl and 1NaCl: 3KCl and then stored at 4°C for 56 days was investigated by Ayyash and Shah (2010). No significant difference between chemical composition, lactic acid bacterial count, proteolysis and pH values of control and experimental cheeses at the same storage period was detected.

Increase in production of small peptides and amino acids as a result of excessive proteolysis due to reduction of salt content leads to more compact and dense microstructure. The compactness of cheese matrix results in harder and less cohesive and springy structure of cheese which in turn affects the overall quality (Gunasekaran and Ak, 2003).

An understanding of the impact of change in milk formulation and various steps in manufacturing of low salt containing Cheddar cheese are required to prepare cheeses having healthy profile with acceptable quality. The objective of this study was to investigate the effect of changes in draining pH, rennet concentration, and casein-to-fat ratio on texture and microstructure of salt-reduced Cheddar cheese during storage at 9±0.5°C for 180 days.

Materials and Methods

Experimental design

Factors including casein/fat ratio of the milk (0.6, 0.7 and 0.8), pH at drainage (6.2, 5.9 and 5.6) and rennet concentration (0.1 and 0.3 ml per L milk) were adjusted. Accordingly, 18 experimental cheeses (3 C/F ratio × 3 pH levels × 2 rennet concentration) were made in duplicates. Table 1 presents the code of each experimental cheese. Every week one experimental cheese was made in duplicate. To ensure that no significant variation come from milk composition, the composition of the milk (Protein = 3.2%, Fat = 3.6%, pH = 6.70) of each trial was measured.

Cheese making

The experimental cheeses were prepared according to Kosikowski (1977) with some modifications. Pasteurized bovine skim milk and cream were purchased from local market and casein/fat (C/F) ratio was standardised to 0.6, 0.7 or 0.8 followed by homogenisation (HST Homogeniser HL3, Unipulse Pty. Ltd, Victoria, Australia) at 50 and 250 bar at 50°C and the milk was kept overnight at 4°C. The following day, the milk was transferred to cheese vat (25 L) and tempered at 32°C for 30-40 min. A solution of 10% calcium chloride (2.5 ml per litre of milk) was added to the milk prior to addition of commercial freeze-dried Cheddar cheese culture (0.25% w/v; R-704 Chr. Hansen, Bayswater, Victoria, Australia), containing *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*. After 35 min, a 0.1% or 0.3% diluted single strength chymosin (CHY-MAX® M, Chr. Hansen) was added followed by 2 min of rigorous mixing. After 35-40 min, the curd was cut into 1 cm^3 cubes using cheese wire knives and cooked at 38°C until pH dropped to 6.2, 5.9 and 5.6. Whey was drained and curds were cheddared at 38°C until pH reached to 5.3. The cheddared curds were milled and salted with NaCl (1.5% w/w) followed by mellowing for 10 min. The salted curds were transferred to 2.5 kg capacity molds and pressed at a pressure of 0.024124 bar (0.0246 kg/cm^2) overnight at room temperature. Pressed cheese samples were vacuum packaged in oxygen barrier bags (Collinsons Pty. Ltd., Fawkner, Australia) using Multivacs vacuum packaging system (Multivac Sepp Haggenmuller, Wolfertschwenden, Germany), and ripened at 9.0 ±0.5°C for 180 days. Sampling was performed at day 0, 60, 120 and 180 of storage. All the following analyses were carried out in duplicates.

Table 2. Composition (dry matter basis) of reduced salt (1.5% w/w of curd) Cheddar cheeses made with different draining pH, C/F ratio, and rennet concentrations at day 0 of storage (mean± SE of 4 replicates).

Treatments	Dry matter %	Protein %	Fat%	Ash%
A	62.94 ± 0.23^{a}	38.43 ± 0.50^{efg}	52.80 ± 0.10^{bc}	4.39 ± 0.03^{ab}
B	62.89 ± 0.10^{a}	37.86 ± 0.19^{g}	52.82 ± 0.06^{b}	4.41 ± 0.05^{ab}
C	64.22 ± 0.20^{b}	38.38 ± 0.32^{cdef}	51.83 ± 0.22^{fgh}	3.74 ± 0.04^{f}
D	63.71 ± 0.16^{b}	38.47 ± 0.25^{defg}	52.34 ± 0.22^{fgh}	3.83 ± 0.14^{ef}
E	63.81 ± 0.24^{b}	40.41 ± 0.46^{b}	51.92 ± 0.22^{cd}	4.06 ± 0.02^{cd}
F	63.82 ± 0.35^{b}	39.22 ± 0.65^{bcd}	52.04 ± 0.18^{fg}	3.76 ± 0.10^{f}
G	64.89 ± 0.17^{c}	37.64 ± 0.53^{defg}	51.23 ± 0.28^{ab}	4.28 ± 0.04^{ab}
H	65.08 ± 0.12^{cd}	36.91 ± 0.23^{efg}	51.01 ± 0.05^{ab}	4.29 ± 0.04^{ab}
I	65.95 ± 0.29^{fg}	37.95 ± 0.75^{efg}	50.37 ± 0.15^{ef}	3.92 ± 0.11^{cd}
J	65.54 ± 0.28^{def}	37.85 ± 0.69^{cde}	50.73 ± 0.08^{cd}	3.99 ± 0.03^{c}
K	66.01 ± 0.12^{fg}	40.77 ± 0.62^{a}	50.17 ± 0.17^{fgh}	4.01 ± 0.09^{bc}
L	66.31 ± 0.25^{g}	38.78 ± 0.37^{b}	50.13 ± 0.25^{gh}	3.73 ± 0.13^{g}
M	65.14 ± 0.19^{cd}	36.77 ± 0.49^{fg}	51.14 ± 0.19^{ab}	4.35 ± 0.05^{a}
N	65.36 ± 0.10^{cde}	36.87 ± 0.48^{efg}	50.86 ± 0.22^{a}	4.35 ± 0.03^{a}
O	66.11 ± 0.13^{fg}	37.50 ± 0.31^{cde}	50.40 ± 0.13^{de}	3.90 ± 0.09^{cde}
P	65.93 ± 0.09^{efg}	37.47 ± 0.35^{cdef}	50.42 ± 0.22^{de}	3.88 ± 0.10^{cde}
Q	66.25 ± 0.24^{g}	38.29 ± 0.39^{bc}	50.17 ± 0.18^{h}	3.72 ± 0.07^{def}
R	65.90 ± 0.27^{efg}	38.53 ± 0.95^{bc}	50.48 ± 0.12^{fgh}	3.59 ± 0.02^{f}

$^{a\text{-}c}$ Means in each column with different letters are significantly different ($P < 0.05$).

Chemical composition

Compositional analysis was performed according to Association of Official Analytical Chemists methods (AOAC International, 1995). In brief, moisture was determined by the 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 mixed 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 quadruplicate.

Texture profile

The texture profile was obtained according to method of Halmos *et al.* (2003) with some modifications. Cheese cylinders (30 mm height × 20 mm diameters) were cut from the center of experimental cheese blocks. Hardness, cohesiveness, and adhesiveness were measured using Texture Analyzer model TA-XT2 (Stable Microsystem, Surrey, UK). Samples were compressed to 30% of their original height. Double-compression was achieved and the data were collected using Texture Exponent software.

Microstructure

Environmental Scanning Electron Microscopy (ESEM) was used to monitor the microstructure of cheeses as described by Sheibani *et al.* (2015). Briefly, 0.5 cm^3 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.

Statistical analysis

Full factorial design was applied to carry out the analysis of all parameters at each storage time. The General Linear Model was applied to examine effects of pH, C/F ratio and rennet concentration at preset significance level of 5% at each storage time. Least significant difference (Fisher`s test) was conducted to test the significance difference between the means of all 18 experimental cheeses at each storage period ($P<0.05$).

Results and Discussion

Chemical composition

The results of chemical composition (based on dry matter) of the experimental Cheddar cheeses made with different draining pH, C/F ratio and rennet concentrations at day 0 of storage are presented in Table 2. Dry matter of all cheeses made with pH 6.2 were significantly lower ($P < 0.05$) compared with those made with pH 5.9 and 5.6. Increase in C/F ratio significantly ($P < 0.05$) increased the protein contents at the Ash contents in cheeses made with 0.6 C/F ratio were higher ($P < 0.05$) compared with other cheeses at the same pH level (Table 2).

Dry matter contents had similar trend to protein content in cheeses with higher C/F ratio due to more expulsion of water (Lawrence *et al*., 2004). The drop in pH at drainage step from 6.2 to 5.6 caused a significant rise in dry matter contents due to high whey expulsion occurred as a result of developing acidity during cooking step. Drop of pH (from ~ 6.5 to 5.3) triggers a decrease in the negative charge of the casein micelles due to titration of negative charges with H^+ ions. Further reduction in pH and approaching isoelectric point of casein micelles

Table 3. Texture parameters of experimental Cheddar cheeses made with different draining pH, C/F ratio, and rennet concentrations during 180 days of storage at 9±0.5°C (values are average of 4 replicates, SEM = Standard error of the mean)

Treatment	Hardness				Cohesiveness				Adhesiveness			
	Day0	Day60	Day120	Day180	Day0	Day60	Day120	Day180	Day0	Day60	Day120	Day180
A	26.5^{defg}	54.7^{bcd}	49.4^{bcde}	34.9^{defg}	0.90^{a}	0.63^{a}	0.68^{abc}	0.77^{a}	0.14^{a}	0.11^{a}	0.10^{abc}	0.07^{de}
B	24.5^{def}	25.1^{def}	24.5^{defg}	23.9^{gh}	0.88^{ab}	0.39^{bcde}	0.67^{ab}	0.74^{ab}	0.13^{cdef}	0.08^{b}	0.04^{c}	0.02^{e}
C	16.1^{efg}	51.8^{bcd}	42.6^{cdef}	36.1^{defg}	0.88^{ab}	0.41^{bcde}	0.42^{de}	0.63^{abcdef}	0.13^{ef}	0.10^{b}	0.05^{c}	0.10^{de}
D	12.2^{g}	27.1^{ef}	26.1^{fg}	22.9^{g}	0.86^{ab}	0.45^{abcd}	0.58^{abcd}	0.75^{def}	0.11^{cdef}	0.07^{b}	0.08^{abc}	0.13^{de}
E	35.5^{bcd}	73.0^{a}	60.7^{bc}	43.1^{cdef}	0.71^{de}	0.49^{abc}	0.59^{abcd}	0.67^{a}	0.10^{ef}	0.07^{b}	0.06^{bc}	0.32^{ab}
F	19.1^{efg}	49.9^{bcde}	32.8^{g}	20.8^{f}	0.67^{e}	0.31^{cde}	0.41^{de}	0.58^{cdef}	0.12^{def}	0.10^{ab}	0.09^{a}	0.18^{bcd}
G	35.6^{bcd}	55.6^{bcde}	52.8^{bcde}	43.0^{cdef}	0.67^{e}	0.42^{bcde}	0.62^{abc}	0.72^{bcdef}	0.07^{ef}	0.07^{ab}	0.07^{bc}	0.02^{e}
H	24.8^{defg}	41.0^{cdef}	28.2^{fgh}	26.9^{efg}	0.66^{e}	0.35^{cde}	0.59^{abcd}	0.66^{def}	0.10^{ab}	0.10^{ab}	0.09^{bc}	0.17^{bcd}
I	16.6^{efg}	53.2^{bcd}	46.4^{cdefg}	37.4^{defgh}	0.64^{e}	0.62^{a}	0.68^{ab}	0.73^{ab}	0.06^{ab}	0.05^{ab}	0.02^{ab}	0.15^{cde}
J	12.5^{fg}	27.3^{def}	26.9^{h}	26.6^{fg}	0.66^{e}	0.47^{abc}	0.55^{bcd}	0.62^{abc}	0.08^{ab}	0.07^{b}	0.06^{abc}	0.20^{abcd}
K	36.5^{bcd}	80.6^{a}	71.2^{ab}	53.4^{bcde}	0.50^{f}	0.26^{e}	0.28^{e}	0.34^{ef}	0.08^{ab}	0.04^{b}	0.05^{abc}	0.11^{de}
L	29.2^{cde}	76.7^{a}	39.6^{cdef}	32.8^{def}	0.52^{f}	0.27^{abcd}	0.76^{a}	0.82^{abc}	0.10^{bcde}	0.10^{ab}	0.08^{abc}	0.34^{a}
M	45.2^{bc}	62.4^{ab}	54.3^{bcd}	48.1^{bc}	0.67^{e}	0.24^{e}	0.44^{cde}	0.61^{bcdef}	0.07^{ab}	0.06^{ab}	0.04^{abc}	0.10^{de}
N	43.7^{bcde}	50.1^{ab}	35.8^{efgh}	32.0^{defg}	0.66^{e}	0.27^{de}	0.50^{bcd}	0.56^{def}	0.09^{abcd}	0.07^{ab}	0.06^{bc}	0.18^{bcd}
O	21.2^{ef}	59.6^{abc}	44.9^{bcde}	43.8^{cdef}	0.73^{de}	0.34^{cde}	0.44^{cde}	0.53^{ef}	0.04^{f}	0.05^{ab}	0.05^{ab}	0.18^{bcd}
P	14.0^{fg}	35.8^{def}	29.4^{fgh}	23.9^{gh}	0.69^{e}	0.31^{cde}	0.36^{e}	0.41^{f}	0.06^{cdef}	0.05^{ab}	0.03^{abc}	0.29^{abc}
Q	53.3^{a}	81.4^{a}	70.3^{ab}	64.7^{b}	0.67^{e}	0.54^{ab}	0.56^{cd}	0.69^{abcd}	0.02^{ef}	0.02^{b}	0.04^{abc}	0.09^{de}
R	38.3^{abcd}	59.0^{bc}	61.2^{abc}	61.0^{abc}	0.76^{de}	0.34^{cde}	0.36^{e}	0.56^{abcde}	0.04^{f}	0.04^{b}	0.02^{abc}	0.09^{de}
SEM^{2}	2.82	4.16	4.05	4.02	0.019	0.022	0.027	0.017	0.006	0.014	0.016	0.017

$^{a-h}$ Means in each column with different letters are significantly different ($P < 0.05$) at same storage period

(pH 4.6) causes more reduction in negative charges of casein micelles and increase in hydrophobicity which in turn enhances aggregation of casein and thus more expulsion of whey (Fox *et al*., 2000, Fox *et al*., 2004). Our observations are in accordance with those of Tunick *et al.* (2007) and Law and Tamime (2010) who reported that the increase in the time between cutting the curd and whey drainage leads to more discharge of whey out of the curd due to acid development. This increase in acidity during cheese making caused more loss of colloidal calcium phosphate (CCP) from curd which in turn decreased the total ash content in cheeses (Roefs *et al*., 1985).

Hardness of cheese

The results of hardness of the all experimental cheeses made with different pH, C/F ratio and rennet concentration after storage of 180 days are presented in Table 3. Regardless of pH and rennet, cheeses made with C/F ratio 0.8 had higher ($P < 0.05$) hardness compared with 0.7 and 0.6 which were almost similar. In general, hardness of all experimental cheeses tended to increase slightly during storage from day 0 to day 60 and then decreased significantly ($P < 0.05$) until end of storage (Table 3). Generally, cheeses made with 0.3 ml/L rennet had lower hardness compared with 0.1 ml/L.

Texture parameters are influenced by chemical composition of cheeses, manufacturing procedure, and ripening conditions (Lucey *et al*., 2003). In this study, we believe that texture parameters were influenced by the treatments during the first weeks of ripening. Afterwards, the main impact on texture profile was a result of ripening process. Therefore, the higher hardness of cheeses made with 0.8 C/F ratio may be attributed to higher dry matter(less moisture) and higher protein contents of these cheeses. This is in agreement with Bryant *et al.* (1995) and Ong *et al.* (2013) who reported that reduction in fat content of Cheddar cheese (increase in protein content) enhanced the hardness of cheese. It has been reported that higher moisture content in cheese leads to softer cheese body (Hennelly *et al*., 2005). It has been noticed that cheeses made with lower pH had generally higher hardness. This may be attributed to the demineralization upon pH drop to 5.9 and 5.6. Hassan and Lucey (2001) reported that Ca^{2+} interaction with casein in Cheddar cheeses decreased from ~64% to ~56% during ripening as a result of pH decrease. The reduction in pH decreases the CCP in cheese which in turn decrease the electrostatic repulsion between caseins resulting in a harder cheese texture (Lucey *et al*., 2003). The impact of higher rennet concentration on hardness was low which may be due to higher rate of protein breakdown occurred in cheese as a result of higher rennet residues remained in cheeses. This may explain the lower hardness profile of cheeses made with 0.3 ml/L rennet compared with 0.1 (Table 3).

Cohesiveness of cheese

Table 3 presents the results of cohesiveness of the all experimental cheeses made with different pH, C/F ratio and rennet concentration during storage of 180 days. Cohesiveness in experimental cheeses decreased significantly ($P < 0.05$) during 60 days of storage and afterwards showed increasing trend. In general, cheeses made with a drainage pH of 6.2

showed higher cohesiveness compared with pH 5.9 and 5.6 from day 60 onwards. As can be seen from Table 3, cohesiveness of cheeses made with different rennet concentration, regardless of pH and C/F ratio, remained unchanged.

Cohesiveness is defined as the strength of the internal bonds to maintain cheese body from rupture when biting completely through the cheese (Gunasekaran and Ak, 2003, O'Callaghan and Guinee, 2004). The drop of pH before drainage increases the demineralization of curd which in turn decreases the CCP in casein micelles (Lucey *et al.*, 2003). Within the casein micelles, casein molecules are held together mostly by hydrophobic interactions and colloidal calcium phosphate (CCP) crosslinks (Fox and Brodkorb, 2008). These CCP links are dissolved with the decrease in pH and therefore caseins may be liberated throughout the serum phase (Pyne and McGann, 1960, Dalgleish and Law, 1989). On the other hand, decrease in pH prevents liberated caseins from movement (Dalgleish and Law, 1989). Thus, no separation of caseins occurs. Moreover, the reduction in CCP decreases electrostatic repulsion between caseins which in turn increases the association between casein molecules (Lucey and Singh, 1997) in cheese and thereby increases hardness and decreases cohesiveness. This may explain the decrease in cohesiveness in our cheeses with lower pH value (5.9 and 5.6) over the storage which

Hardness has an opposite trend to cohesiveness. Increase in hardness creates more brittle and less cohesive cheese texture (Fox *et al.*, 2000, Gunasekaran and Ak, 2003, Maldonado *et al.*, 2013). On the other hand, increase in protein content in cheeses as a result of the increase of C/F ratio from 0.6 to 0.8 may explain the decrease hardness and thus increase in cohesiveness (Bryant *et al.*, 1995). The increase in cohesiveness after 60 days of ripening may be resulted from excessive hydration of casein due to low salt concentration in our cheese. It is well established that excessive casein hydration results in a softer cheese texture and therefore greater cohesiveness (Fox and McSweeney, 1996, Fox *et al.*, 2000, Upadhyay *et al.*, 2004).

Adhesiveness of cheese

The results of adhesiveness of all experimental cheeses made with different pH, C/F ratio and rennet concentration during storage of 180 days are presented in Table 3. In general, adhesiveness increased significantly ($P < 0.05$) at day 60 of storage compared with day 0 in all experimental cheeses except Cheddar cheeses made with pH 5.9, C/F ratio 0.8 and rennet 0.1. Analysis of variance showed that storage and C/F ratio*rennet*pH had significant effect on adhesiveness. Generally, adhesiveness showed decrease as pH dropped from 6.2 to 5.6.

Adhesiveness is defined as stickiness of sample in the mouth throughout mastication or the amount of force required to remove the cheese from the palate during eating (Gunasekaran and Ak, 2003). Increase in amount of protein which alters the protein matrix, making the cheese matrix more compact and therefore less adhesive. A similar trend was observed by Bryant *et al.* (1995) who stated that lower values of adhesiveness was observed in low-fat cheeses ripened for up to 4 months. It can explain the decrease in adhesiveness of our cheese made with higher C/F ratio (0.8). During ripening, in addition to proteolysis, moisture also move out of the protein matrix resulting in a more homogeneous matrix which in turn decreases the adhesiveness over storage (Irudayaraj *et al.*, 1999). This can be accelerated in salt-reduced cheeses due to higher reduction of pH which enhances moisture discharge from cheese matrix and therefore less adhesiveness (Fox *et al.*, 2000, Fox *et al.*, 2004). The increase of adhesiveness in cheeses at the end of the storage (day 180) may have resulted from activity of bacterial proteolytic enzymes (cell wall proteinase and intracellular peptidases) which breakdowns the peptides into small amino acids in cheese matrix and thus forming a soft and sticky cheese (Fox *et al.*, 2000, Parente and Cogan, 2004).

Microstructure of Cheese

Microstructure images of 18 experimental Cheddar cheeses at day 0 and 180 of storage are presented in Figure 1. Storage time affected the microstructure of all cheeses. The comparison between images at day 0 and day 180 cheese samples show that microstructure became dense and compact with small gaps due to presence of more soluble and swollen proteins (Rowney *et al.*, 2004). Similar observations were noted in the texture parameters (Table 3) which showed increase in hardness. Moreover, at day 180, images show that porosity and cavities vanished and cheese samples became more homogenous compared with day 0. As can be seen from Figure 1, microstructures of all cheeses made with pH 5.6 were denser and compact compared with those made with pH 5.9 and 6.2. At the end of storage time (Day 180), the differences between cheese samples disappeared and it was difficult to notice the effects of C/F ratio, pH and rennet concentration.

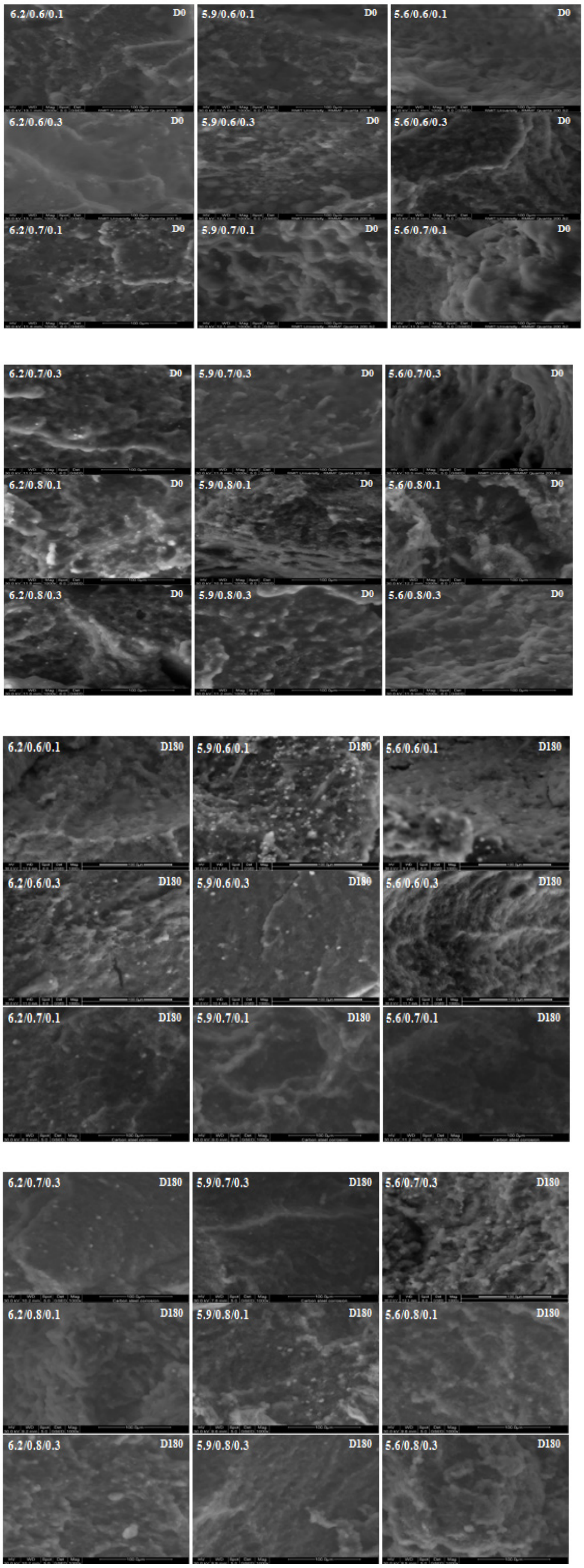

Figure 1. Microstructure of experimental Cheddar cheeses made with different draining pH, C/F ratio, and rennet concentrations at day 0 (D0) and 180 (D180) of storage at 9±0.5ºC

Conclusion

The increase in C/F ratio and rennet concentration has influenced the texture of cheeses due to higher proteolysis. Hardness increased in all cheeses at early stages of ripening (up to day 60) and then gradually decreased by end of storage (day 180). Adhesiveness of all cheeses decreased significantly over the storage period. Cohesiveness of all cheeses showed an opposite trend to hardness. It showed decreasing trend towards day 60 of storage and then slightly increased by the end of storage time. Reducing the pH caused an increase in the rate of proteolysis which has direct impact on texture. Ash content of cheese was also lower at lower pH which in turn influences the proteolytic activity and texture. Reduction of pH increased the demineralization of colloidal calcium phosphate (CCP) which enhanced an association among casein molecules. Therefore, cheeses made with lower pH had more dense and compact structure and narrower voids compared with those made at higher pH. Consideration should be given in future to expand the range of rennet concentration to clearly understand its role on texture and microstructure of cheese.

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