Texture, flavor, and sensory quality of buffalo milk Cheddar cheese as influenced by reducing sodium salt content

M. A. Murtaza,*¹ N. Huma,† A. Sameen,† M. S. Murtaza,† S. Mahmood,* G. Mueen-ud-Din,* and A. Meraj*

*Institute of Food Science and Nutrition, University of Sargodha, Sargodha-40100, Pakistan

†National Institute of Food Science and Technology, University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

The adverse health effects of dietary sodium demand the production of cheese with reduced salt content. The study was aimed to assess the effect of reducing the level of sodium chloride on the texture, flavor, and sensory qualities of Cheddar cheese. Cheddar cheese was manufactured from buffalo milk standardized at 4% fat level by adding sodium chloride at 2.5, 2.0, 1.5, 1.0, and 0.5% (wt/wt of the curd obtained). Cheese samples were ripened at 6 to 8°C for 180 d and analyzed for chemical composition after 1 wk; for texture and proteolysis after 1, 60, 120, and 180 d; and for volatile flavor compounds and sensory quality after 180 d of ripening. Decreasing the salt level significantly reduced the salt-in-moisture and pH and increased the moisture-in-nonfat-substances and water activity. Cheese hardness, toughness, and crumbliness decreased but proteolysis increased considerably on reducing the sodium content and during cheese ripening. Lowering the salt levels appreciably enhanced the concentration of volatile compounds associated with flavor but negatively affected the sensory perception. We concluded that salt level in cheese can be successfully reduced to a great extent if proteolysis and development of off-flavors resulted by the growth of starter and nonstarter bacteria can be controlled.

Key words: Cheddar cheese, sodium salt, texture, flavor, sensory quality

INTRODUCTION

Cow milk is under focus in most of the studies on milk, although milk of other species, such as sheep, goats, camels, and buffaloes, has nutritional potential in the human diet in different parts of the world (Ménard et al., 2010). Globally, Cheddar-type cheese is manu-

factured from cow milk; however, buffalo milk is at the top in milk production of Pakistan. India and Pakistan together produce more than 80% of the world's buffalo milk (Murtaza et al., 2012). It is more nutritious, being richer in lactose, fat, protein, calcium, magnesium, inorganic phosphate, and vitamins compared with cow milk (Ahmad et al., 2008). On account of its composition, buffalo milk offers exceptional prospects for the manufacture of different milk products (Murtaza et al., 2008).

Cheddar is a nutritious, hard type of cheese. It endures substantial modifications on account of ripening, which is the consequence of glycolysis, lipolysis, and proteolysis (Farkye 2004; Murtaza et al., 2012). These microbial and biochemical reactions are the foundation for development of flavor, texture, and sensory perception of cheese (Lucey and Singh 2003; Smit et al. 2005; Murtaza et al., 2013).

Sodium salt is extensively and effectively used as a preservative and flavor enhancer. Its concentration is imperative for physicochemical and sensory characteristics of dairy foods, particularly cheese (Mendil, 2006). Sodium chloride affects the compositional profile of cheese as well as water activity, microbial growth and activity of enzymes, curd syneresis, and solubility and hydration of protein during the course of ripening, leading the synchronized improvement in flavor and texture (Johnson et al., 2009; Murtaza et al., 2012; Rulikowska et al., 2013).

Consuming sodium contributes to the progression of hypertension, a predecessor for disorders, such as cardiovascular problems, leading to increased risk of heart attacks or strokes in many people (Cotugna and Wolpert, 2011; Grummer et al., 2013). Traditionally, the level of sodium salt in Cheddar-type cheese is about 2.0%. However, because of its adverse health effects, great demand exists to reduce the salt levels in cheese manufacturing (Rulikowska et al., 2013).

Keeping the particulars mentioned above in vision, this study was aimed to assess the effect of reducing the level of sodium chloride on water activity, proteolysis, texture, flavor, and sensory quality of Cheddar cheese prepared from buffalo milk.

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¹Corresponding author: anjum_ft@yahoo.com or mian.anjum@uos.edu.pk

MATERIALS AND METHODS

Manufacturing and Ripening of Cheddar Cheese

Buffalo milk was obtained from the dairy farm at the University of Agriculture (Faisalabad, Pakistan) and standardized to a 4.0% fat level. Cheddar cheese was manufactured using commercially available freeze-dried mesophilic starter cultures (*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis*) and by adding sodium chloride at 2.5, 2.0, 1.5, 1.0, and 0.5% (wt/wt of the curd obtained), following the standard procedure described by Murtaza et al. (2012). Cheese samples were ripened at 6 to 8°C for 180 d.

Chemical Composition

One-week-old cheese was grated and analyzed for salt (NaCl), moisture, fat, protein, ash, pH, salt-in-moisture, fat-in-DM, and moisture-in-nonfat-substances in triplicate samples. Salt (by titration), moisture (oven drying), fat (Babcock method), protein (Kjeldahl method), and ash (ignition) content were determined as described by Kirk and Sawyer (1991). The pH was measured with a digital pH meter (Ong et al. 2007).

Proteolysis

Proteolysis in cheese samples was assessed at 60-d intervals during ripening using HPLC, following the procedure detailed by Hickey et al. (2006) and modified by Rulikowska et al. (2013). Five grams of cheese was homogenized in 20 mL of 0.1 M citrate buffer at pH 3.6 at 40°C for 1 h to separate the fat. The whole suspension was centrifuged at $3,000 \times g$ for 30 min at 4°C. The resulting pellet (100 mg) was dissociated in 5 mL of disassociating solution and held in a water bath at 40°C for 1 h with periodic mixing. This mixture was then centrifuged at $20,000 \times g$ for 10 min at room temperature and filtered through a nylon 0.45-mm syringe filter. An aliquot of 20 mL was injected into a Shimadzu liquid chromatograph (LC-10 AT VP series; Shimadzu Corp., Kyoto, Japan) with a UV-visible detector at 214 nm (Shimadzu Corp.). Separation was achieved using a gradient of 2 mobile phases: (A) 90% water and 10% acetonitrile with 0.1% trifluoroacetic acid and (B) 90% acetonitrile and 10% water with 0.1% trifluoroacetic acid at a flow rate of 0.8 mL/min. The gradient was 75% A for 12 min, reduced to 51% A over 31 min, a further reduction to 20% A over the next 13 min, held at 20% A for another 3 min, and then an increase to 75% A over 3 min and held at this concentration for a further 2 min; the total run time was 54 min. The column was a C4 Jupiter 5 mm 300 A column (Shimadzu Corp.).

Texture Profile Analysis

The effect of salt addition on the texture of Cheddar cheese was evaluated after 1, 60, 120, and 180 d of ripening by performing the texture profile analysis of the cheese samples on a texture analyzer (Stable Micro Systems Ltd., Godalming, UK), using compression plate probe P-75 as described by O'Mahony et al. (2005).

Volatile Flavor Compounds

The volatile compounds of Cheddar cheeses with varying salt levels were isolated using a dynamic headspace analyzer coupled with a gas chromatograph (Agilent Technologies Ireland Ltd., Little Island, Co. Cork, Ireland). The samples were prepared using the procedure of Rulikowska et al. (2013). The trapped volatiles were injected to the column of the gas chromatograph equipped with a capillary column. Mean peak areas were calculated for individual compounds using the Chemstation software and standard library (Agilent Technologies Ireland Ltd.). Volatiles analyses were performed in triplicate after 180 d of ripening.

Sensory Evaluation

Cheddar cheese samples was evaluated for sensory characteristics (odor, flavor, texture, mouthfeel, and overall acceptability) after 180 d of ripening on a hedonic rating scale (0–9) by a panel of 10 assessors drawn from faculty members and postgraduate students, as described by Awad et al. (2004). The samples awarded scores less than 5 were considered as of low quality.

Statistical Analysis

Each treatment (salt level) of cheese was produced in triplicate ($n = 3$). The results obtained from different parameters (no. of factors 1 and 2) were subjected to statistical analysis by ANOVA under a completely randomized design, as described by Steel et al. (1997).

RESULTS AND DISCUSSION

Cheese Composition

The compositional profile of Cheddar cheese is illustrated in Table 1. The cheese samples were significantly different in salt concentration as per addition of salt during manufacturing. Notably the reduction in sodium chloride content significantly decreased the losses of salt in whey. The noncomparative increase in salt uptake might be because of the associated variation in level of whey discharged from the curd after salt addition. As

Figure 1. Water activity (means of triplicate determinations ± SD) in cheese with reduced sodium salt content.

expected, salt concentration significantly affected the cheese composition. These findings are in agreement with those reported previously (Schroeder et al., 1988; Rulikowska et al., 2013). Overall, the reduction in salt levels resulted in decreasing the protein, fat, ash, and salt-in-moisture contents and the pH, and increasing the moisture, fat-in-DM, and moisture-in-nonfat-substances contents. As per increase in moisture, the fat and protein contents decreased because of attenuation effects. The pH of cheese samples ranged from 4.88 to 5.12 and decreased with decreased the sodium salt content. The pH of cheese is reliant on starter cultures, acidification, the pH when the whey is drained, and the salt-in-moisture content (Rulikowska et al., 2013).

Similar to the starter cultures, the acidification rate and pH at drainage were alike for all samples; the pH was influenced by salt-in-moisture content.

Water Activity

The mean water activity (a_w) in cheese was significantly ($P < 0.05$) influenced by the reduction in salt content (Figure 1). The a_w is affected by the moisture and solute contents in cheese (Guinee and Fox, 2004). The a_w increased with reduced sodium salt content as a result of increases in the moisture-in-nonfat-substances content. Cheese samples having 2.5 and 2.0% sodium chloride content had a statistically nonsignificant ($P >$

Table 1. Compositional profile of Cheddar cheese

Item	Salt (%) in cheese samples				
	2.5	2.0	1.5	1.0	0.5
Composition (%)					
Salt	1.84 ^a	1.42 ^b	1.22 ^c	0.85 ^d	0.45 ^e
Moisture	37.98 ^d	38.14 ^{cd}	38.88 ^c	39.84 ^b	40.90 ^a
Fat	31.45	31.22	30.94	30.60	30.32
Protein	25.06 ^a	24.82 ^{ab}	24.52 ^b	24.32 ^{bc}	24.18 ^c
Ash	4.02 ^a	3.58 ^b	3.36 ^{bc}	3.05 ^c	2.68 ^d
Salt-in-moisture	4.52 ^a	4.08 ^{ab}	3.60 ^b	2.58 ^c	1.30 ^d
Fat-in-DM	50.64 ^d	51.20 ^{cd}	51.70 ^c	52.14 ^b	52.90 ^a
Moisture-in-nonfat-substances	56.55 ^d	57.03 ^{cd}	58.27 ^c	59.91 ^b	60.93 ^a
pH	5.12 ^a	5.04 ^b	4.98 ^{bc}	4.95 ^{bc}	4.88 ^c

^{a-e}Values with the same superscript letters within a row do not differ significantly at a significance level of 5%.

Figure 2. Proteolysis in Cheddar cheese containing 0.5, 1.0, 1.5, 2.0 or 2.5% salt. (a) Water-soluble nitrogen as a percentage of total nitrogen; (b) total free amino acid content.

0.05) difference in a_w , probably because of the nonsignificant difference in protein content, which influences the water-holding capacity of the cheese. The variations in a_w significantly influenced proteolysis because the microbial and enzymatic activities are associated with a_w (Choisy et al., 2000; Lawrence et al., 2004).

Proteolysis

The decrease in sodium salt content significantly increased proteolysis (Figure 2a), as anticipated based on

the reported literature (Kelly et al., 1996; Rulikowska et al., 2013). The escalation in proteolysis might be primarily the result of more bacterial and enzymatic activities due to the higher moisture and moisture-in-nonfat-substances contents and greater a_w (Guinee, 2004). In general, significant differences ($P < 0.05$) were found for mean values of water-soluble nitrogen (pH 4.6) at each point during ripening between the cheese samples. The level of primary proteolysis was considerably higher for samples having lower salt concentration and pointedly lower for cheese with higher

Figure 3. Texture profile (means of triplicate determinations $\pm$ SD) of Cheddar cheese as influenced by reduced salt content and ripening for up to 180 d. (a) Cheese hardness; (b) cheese toughness; (c) cheese crumbliness.

Table 2. Volatile flavor compounds in Cheddar cheese (average peak areas in arbitrary units $\times 10,000$)

Volatile flavor compound	Salt (%) in cheese samples				
	2.5	2.0	1.5	1.0	0.5
Alcohol					
Ethanol	88,345	99,723	74,982	76,025	80,452
Propanol	1,550	1,892	8,550	20,855	60,826
1-Butanol	105	112	138	650	1,890
2-Butanol	1,856	2,508	10,520	58,470	75,082
Hexanol	305	682	220	185	274
Heptanol	10	28	52	86	148
Aldehyde					
Pentanal	412	404	395	428	400
Hexanal	198	173	140	122	104
Heptanal	76	81	88	105	156
Octanal	36	40	51	69	82
Ketone					
Butanone	8,210	14,728	17,090	19,453	24,028
Pentanone	724	480	210	136	98
Hexanone	154	182	170	306	582
Heptanone	422	380	316	256	176
Ester					
Ethyl acetate	2,698	2,840	2,903	3,276	3,687
Ethyl butanoate	1,892	1,863	1,906	1,875	1,952
Ethyl hexanoate	174	188	200	222	264
Sulfur compounds					
Dimethyl disulfide	512	568	670	792	910
Dimethyl trisulfide	12	18	58	70	78

salt content. The greater degree of primary proteolysis in Cheddar cheese having less sodium chloride added was established by greater breakdown of caseins by proteases, which is in agreement with Kelly et al. (1996) and Mistry and Kasperson (1998).

Regarding the free amino acids, the reduction in the salt content considerably increased the levels (Figure 2b). Significant differences ($P < 0.05$) were found in the total free amino acids content at each salt concentration added during the early phases of ripening. These differences remained throughout the course of ripening; however, they were inconsistent. This might be because of changes in proteolysis (Rulikowska et al., 2013).

Texture Profile Analysis

The texture profile analysis of Cheddar cheese showed that the hardness, toughness, and crumbliness decreased upon reducing the sodium salt concentration and with the process of ripening (Figure 3). The textural parameters were significantly ($P < 0.05$) influenced by the salt levels, ripening time, and their interaction. The cheeses having 0.5 or 1.0% salt content added could be categorized as soft body cheeses compared with the other treatments.

It is recognized that the increased proteolysis and moisture-in-nonfat-substances affect the cheese texture (Mistry and Kasperson, 1998; Guinee, 2004) and result in decreasing the hardness, toughness, and crumbliness.

Moreover, casein hydration was also found to be involved in the development of cheese texture. The salt addition in cheese improves casein hydration as well as the volume of the matrix and solubilization of casein through calcium displacement by sodium ions (Guinee, 2004). Thus, in the present study, the decrease in sodium salt content might have led to a decrease in calcium displacement and reduced casein solubilization, resulting in less casein hydration; a decreased displacement of calcium and reduced casein solubilization, leading to less casein hydration because of the reduced sodium chloride content in cheese was also reported by Rulikowska et al. (2013).

Sodium salt also ultimately controls the textural characteristics indirectly through its influence on cheese pH, which has an effect on starter and enzyme activities (Lawrence et al., 2004).

Volatile Flavor Compounds

About 19 flavor compounds, including 6 alcohols, 4 aldehydes, 4 ketones, 3 esters, and 2 sulfur compounds were detected in volatile analysis of the Cheddar cheeses. All these compounds have previously been detected in Cheddar-type cheese (Singh et al., 2003). Cheese samples having lower sodium salt content were found to have a higher concentration of volatile compounds, as depicted by the average peak areas (Table 2). However, the quantity of ethanol increased upon increasing the

Table 3. Sensory evaluation (scores) of Cheddar cheese¹

Parameter	Salt (%) in cheese samples				
	2.5	2.0	1.5	1.0	0.5
Odor	6.7 ^b	7.8 ^a	6.5 ^b	6.0 ^c	5.2 ^d
Flavor	8.0 ^a	7.6 ^b	6.8 ^c	6.2 ^d	5.0 ^e
Texture	7.4 ^a	7.6 ^a	7.0 ^b	6.5 ^c	5.6 ^d
Mouthfeel	7.5 ^{ab}	7.8 ^a	7.2 ^b	6.5 ^c	6.0 ^d
Overall acceptability	7.6 ^a	7.7 ^a	6.8 ^b	6.3 ^c	5.4 ^d

^{a-e}Values with the same superscript letters within a row indicate that samples do not differ significantly at a significance level of 5%.

¹Sensory evaluation was done on a hedonic rating scale (0–9) by a panel of 10 assessors. Samples awarded scores less than 5 were considered as of low quality.

salt level, as the highest peak area of ethanol was in cheese with 2.5% added salt.

The characteristic properties of all these compounds in cheese flavor are recognized, except the ketones, which are the direct outcome of oxidation of FFA during lipolysis (Reineccius, 1999). These ketones are mostly related to fruit- or ether-like flavors in Cheddar cheese (Singh et al., 2003) and are the precursors of alcohols and aldehydes, which make great contributions to cheese flavor. The aldehydes, alcohols, and ethyl esters are the outcomes of reduction of FFA, amino acid metabolism, and reaction of FFA and alcohols, respectively. The former are found to be linked with oily, fatty, pungent, and soapy flavors (Singh et al., 2003). Sulfur compounds are instigated from the metabolic changes in sulfur-containing amino acids, such as methionine, and are connected with onion-, cabbage-, and garlic-like volatiles (McSweeney and Sousa, 2000; Williams et al., 2001).

The higher concentration of volatiles in cheese with less added sodium salt must be due to the early growth of starter and nonstarter bacteria and enzymatic activity, as salt addition to cheese exerts control on bacterial growth, enzymatic activities, and reactions during ripening; hence, salt addition regulates the development of flavor and aroma (Murtaza et al., 2012). The discrepancies in flavor profiles of cheeses having lower and higher quantities of added salt are likely due to the changes in composition.

Sensory Evaluation

The reduction in salt added greatly influenced the sensory perception of the cheese samples. Statistical analysis of the scores awarded (0–9) showed significant variation ($P < 0.05$) among all samples. The samples having 2.0% added salt were liked and scored the highest (Table 3). Although the volatile flavor compounds had higher concentrations in cheese with lower salt concentrations, the sensory flavor perceptions were the

highest for cheese with 2.5% added salt, with a gradual decrease as salt contents reduced.

Drake et al. (2001) described that “sweetness” is normally inversely associated with “bitterness.” This concurs with the findings of the current study, as bitterness caused by nonstarter bacteria was counteracted by the addition of salt and, hence, a sweet perception resulted. The increased hydrolysis of caseins relates to augmented bitterness, probably due to the decreased aggregation of caseins upon reducing sodium salt content (Guinee and O’Kennedy, 2007).

CONCLUSIONS

We concluded that reducing the sodium chloride content has a substantial effect on cheese composition and proliferation of proteolysis, and increases the a_w and volatile flavor compounds content, but decreases the sensory discernment. Regarding the texture, hardness, toughness, and crumbliness lessened upon reducing the salt content and during the course of ripening. Hence, quality Cheddar cheese can be manufactured with reduced sodium salt content by controlling and monitoring the proteolysis and a_w .

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