Effect of Moderate Pressure Treatments on Microstructure, Texture, and Sensory Properties of Stirred-Curd Cheddar Shreds

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ABSTRACT

A moderate high-pressure processing (HPP) treatment is proposed to accelerate the shredability of Cheddar cheese. High pressure processing (345 and 483 MPa for 3 and 7 min) applied to unripened (1 d old) stirred-curd Cheddar cheese yielded microstructure changes that differed with pressure level and processing time. Untreated and pressure-treated cheese shredded at d 27 and 1, respectively, shared similar visual and tactile sensory properties. The moderate (345 MPa) and the higher (483 MPa) pressure treatments reduced the presence of crumbles, increased mean shred particle length, improved length uniformity, and enhanced surface smoothness in shreds produced from unripened cheese. High-pressure processing treatments did not affect the mechanical properties of ripened cheese or the proteolytic susceptibility of milk protein. It was concluded that a moderate HPP treatment could allow processors to shred Cheddar cheese immediately after block cooling, reducing refrigerated storage costs, with expected savings of over 15 US\$/1000 lb cheese, and allowing fewer steps in the handling of cheese blocks produced for shredding.

(Key words: Cheddar cheese, high-pressure processing, microstructure, shredability)

Abbreviation key: HPP = high-pressure processing, NPN = nonprotein nitrogen, SEM = scanning electron microscopy, TN = total nitrogen, TPA = texture profile analysis, WSN = water-soluble nitrogen.

INTRODUCTION

Cheddar cheese is the most widely consumed cheese in the United States. It is a multifunctional ingredient,

with shredding used by cheese manufacturers to market a value-added product. The same interest in shredded cheese has been observed elsewhere in the world. Shredded Cheddar cheese and other natural cheeses add unique flavor notes to pizzas, salads, and many other recipes. The high cheese production volumes and low prices currently observed in the United States have forced dairy producers to seek alternatives reducing the cost of shredded Cheddar cheese, while at the same time improving quality. Many cheeses could be shredded commercially, but cold-storage costs before a cheese can be shredded are often an economic barrier in furthering market growth. Cheddar cheese requires about 30 d of ripening before shredding, adding refrigerated storage and handling costs to the product. Key attributes improved by ripening include the reduction of crumbs and improvements in surface smoothness, shred mean length, and size uniformity. Other desirable attributes improved by ripening are shred-to-shred piece adhesiveness, visual perception of oiliness, and tactile oiliness after shred handling (McMahon, personal communication, 2001).

Two manufacturing technologies are used in Cheddar cheese production. In traditional or milled-curd technology, blocks of about 25-cm height are piled on top of each other, allowed to rest for about 15 min, and then turned over. This procedure (cheddaring) is repeated 2 or 3 times to reach the desired curd acid level. In the more recently developed stirred-curd approach, curd is not cheddared; instead, drained curd is agitated continuously to reach the desired acid level. The constant agitation of the curd does not allow knitting of curd particles, but the Cheddar cheese obtained has similar composition and properties. Stirred-curd technology is simpler to mechanize than the traditional method (Williams, 1997).

The microstructure of Cheddar cheese can be described as a continuous protein matrix, creating a po-

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rous structure with spaces occupied by fat globules distributed in the casein network (Bryant et al., 1995). The casein network depends on chemical composition, manufacturing process, proteolysis during ripening, and the size and distribution of the fat globules (Lagoueyte et al., 1994; Metzger and Mistry, 1995; Bryant et al., 1995; Sutheerawattananonda et al., 1997). Torres-Mora et al. (1996) were first in observing that high-pressure treatments (275 MPa for 100 s) applied to fresh curd immediately yielded a microstructure similar to aged Cheddar cheese, a finding that was later confirmed by Yang (1999).

Many publications describe microstructure and cheese composition effects on the mechanical and textural properties of cheese (e.g., Lee et al., 1978; Charalambides et al., 1995; Hort and LeGrys, 2000). Nienaber et al. (2000) subjected stirred-curd Cheddar cheese to hydrostatic pressure ranging from 100 to 800 MPa (5 min, 25°C) at different times during ripening. Pressure-treated cheese samples and controls showed a gradual decline in hardness, cohesiveness, gumminess, and chewiness over storage time. Pressurization at 400 MPa resulted initially in hardness reduction, but after 10 wk of storage, hardness, cohesiveness, gumminess, and chewiness of the pressurized cheeses were not different from controls. However, the 800-MPa treated cheese had higher hardness, cohesiveness, gumminess, and chewiness than the controls. Saldo et al. (2000a, 2000b) and Jin and Harper (2002) suggested that high-pressure processing (HPP) accelerates ripening by increasing moisture retention, releasing bacterial enzymes, and increasing enzyme activity while the cheese is under pressure. However, the effect of pressure treatments on aging has been reported to depend on pressure conditions and cheese variety (Messens et al., 1999, 2000; Saldo et al., 2000a).

The growth in shredded Cheddar cheese consumption provides the main justification for this work, and thus the central objective of this study was to determine the feasibility of using HPP to eliminate or reduce the ripening time needed before Cheddar cheese reaches shredability. This had to be achieved without deleteriously affecting the attributes of cheese shreds. Relatively little information has been published on the mechanical properties making cheese blocks suitable for shredding (Apostolopoulos and Marshall, 1994). Also absent in the published literature was a definition of sensory methods that processors could use in the development of consumer-acceptable shredded cheeses. This required developing a sensory evaluation methodology for shredded cheese and the examination of the microstructure and mechanical properties of pressure-treated cheese. Changes in sensory attributes by HPP may be associated with protein pressure-induced dena-

turation and aggregation and changes in proteolytic enzymes. Thus, this study included determination of mechanical, microstructure, and biochemical properties to explain changes induced by pressure treatments and storage time on the sensory properties of shredded pressure-treated cheese.

MATERIALS AND METHODS

Cheese Samples

Fresh cow's milk stirred-curd Cheddar cheese blocks (18.2 kg) provided by Glanbia Foods Inc. (Twin Falls, ID) were collected immediately after cooling and transported refrigerated for pressure processing at the facilities of Avure Technologies, Inc. (Kent, WA). Blocks were cut into small blocks (7 × 3.5 × 18 cm) and vacuum-packed for treatments at 345 and 483 MPa for 3 and 7 min within 24 h of sample collection. Untreated and pressure-treated cheese was placed in storage for ripening at 7°C for further analysis.

Chemical Analysis of Cheese

Pressure-treated and untreated cheese samples were analyzed in triplicate after 1, 6, 13, and 27 d of ripening for moisture, protein, fat, sodium, and pH (Marshall, 1992). Proteolysis throughout storage was monitored using the microKjeldahl method (AOAC, 2000) by measuring total nitrogen (TN), water-soluble nitrogen at pH 4.6 (WSN), and nonprotein nitrogen (NPN) measured after removing proteins with a 12% TCA solution.

Texture Analysis

Cubes of 20 mm, cut using a custom-designed wire cutter to obtain samples of uniform shape and size, were kept in sealed plastic bags to avoid surface drying. Cut samples were held at 4°C for 12 h before performing compression tests using a TA.XT2 Texture Analyzer (Stable Micro Systems, UK). Texture profile analysis (TPA) at 50% was performed at 1 mm/s of crosshead speed with a 25-kg load cell and 50-mm diameter aluminum probe (model P/50). Ten samples for each treatment were analyzed after 1, 6, 13, and 60 d of ripening to calculate hardness, springiness, and cohesiveness values. Equipment availability required the change in the last sampling day for texture analysis.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) analysis was carried out at the OSU Electron Microscopy laboratory. Small pieces (2 to 3 mm), collected 1 d after pressure treatment, were chemically fixed with glutaraldehyde

buffer in refrigerated glass vials. The fixative reagent was then removed and samples were washed for 24 h with 0.0125 M pH 7.2 cacodylate buffer, transferred into 1% osmium tetroxide in fresh cacodylate buffer for 48 h, then passed through 50 and 70% ethanol/water solutions and washed twice with 100% ethanol with a minimum of 4 h per change. Each sample was then placed in an aluminum weighing pan containing liquid nitrogen. Fully frozen samples were pressure fractured, with the resulting pieces collected into biopsy bags (Fisher Scientific 15 to 182 to 506H), stored in absolute ethanol, and then critical-point dried (Anderson, 1951, 1966; DeBault, 1973). Under stereo light microscopy, small pieces of thoroughly fixed fractured cheese were mounted on aluminum planchettes with the fractured surfaces upward and then coated with 200 nm of 60/40 wt % Au/Pd alloy in a Varian VE-10 vacuum evaporator (Varian, Inc. Vacuum Technologies, Lexington, MA).

Sensory Analysis

A descriptive sensory analysis (Lawless and Heymann, 1998; Meilgaard et al., 1999) was performed by a trained panel consisting of 9 subjects (4 males and 5 females) from the OSU Department of Food Science and Technology. During training, panelists were asked to identify and define visual appearance and tactile properties characterizing shredded cheese. Panelists were presented with an array of commercial shredded cheese samples to aid in the development of these terms. Descriptors selected for appearance were visual oiliness, surface smoothness, shred mean length, uniformity of shred length, and amount of crumbles. Other terms selected by the panel were tactile oiliness and shred adhesiveness (Table 1). Panelists marked responses on a 9-point intensity scale anchored with "not" on the left and "very" on the right ends of the scale. Panelists were trained for 2 wk in 6 sessions of 1 h; in the first 2 sessions, the panel determined the terminology and the definition of the 7 descriptors; in the following 2 sessions, panelists suggested standards and also scored them; and the final 2 sessions were used for practice sessions with commercial and pressure-treated samples. All panelists were monetarily compensated for their participation in the study.

During the sensory analysis of experimental samples, panelists evaluated 10 samples per session. Cheeses were shredded using a food processor (Hobart Corp. Troy, OH, model A200) with a front-mounted taper attachment hub (model VS9) and adjustable shredder plate (3/16", model VS9). Then 5 g of cheese was presented in 3-digit random coded plastic sample cups (50 mL) covered with lids to avoid surface drying and held in a constant temperature (4°C) for at least 2 h before

sensory analysis. For sample evaluation, the panelist poured the cup into 15.2-cm diameter white foam plates, with testing performed under white fluorescent light. Napkins were provided to cleanse hands between samples, which was mandatory for panelists. Sample presentation order was randomized across sessions and judges, with sample evaluations conducted at 1, 6, 13, and 27 d after pressure treatment.

Statistical Analysis

All data were analyzed by multivariate ANOVA (MANOVA) using SPSS v.10 (SPSS Inc., Chicago, IL) to inspect the effect of pressure, process time, and storage on the attributes measured instrumentally and sensorially with 2 replications. Significance differences for least square means were determined at 95% significance level. The same software was used to conduct principal component analysis to identify experimental groups.

RESULTS AND DISCUSSION

Cheese Proximate Analysis

Chemical composition determined at 1, 13, and 27 d showed that moisture (38 to 38.1%), fat (33.1 to 33.5%), protein (35.1 to 37.5%), and sodium (194.2 to 199.3 mg/kg) contents were not significantly different among the pressure treatments and did not change with storage time. Similar results were obtained by Capellas et al. (2001), who reported that 100 to 400 MPa treatments did not alter total solids, ashes, fat, and soluble nitrogen.

Sensory Analysis

Values for the sensory descriptors used by the trained panel (Table 1) to evaluate shredded samples from untreated and pressure-treated cheese are summarized in Figure 1. Pressure level, pressure-holding time, and storage significantly affected the shred mean length of all samples. Storage time had more effect on the shred mean length values of untreated samples (controls), which increased with storage (scored 4.8 to 5.7); however, storage time had less effect on pressure-treated samples (Figure 1a). Pressure level in conjunction with processing time affected the mean length of shreds from treated cheese. Pressure-treated samples had longer mean length than controls stored up to 6 d when the shred mean length of treated and control samples were comparable.

Pressure treatments had an obvious effect on the length uniformity of shreds (Figure 1b). Untreated cheese (scored 2.3 to 4.7) yielded shreds that had less

Table 1. Definition of the sensory attributes for shredded Cheddar cheese

Descriptor	Definition	Cheese standards
Appearance descriptors		
Procedure: Observe at 30 cm from the sample and evaluate without considering the presence of crumbles and fines		
Visual oiliness	How shiny the sample is	Parmesan fine shred, 6 oz, Lucerne Sharp Cheddar, 2 lb, Tillamook County Creamery Assoc. Swiss, 2 lb, Lucerne
Smooth surface	How smooth the outside of the strip is	Sharp Cheddar, 2 lb, Tillamook County Creamery Assoc. Mild Cheddar, 2 lb, Lucerne, Mozzarella cheese, low moisture, part skim milk, 2 lb, Lucerne
Mean length	Average length of cheese shreds	Yellow paper shreds (1, 2, and 4 cm)
Uniformity of length	How uniform in length the strips are, not considering presence of crumbles and fines	Medium Cheddar, shredded, 8 oz, Lucerne Sharp Cheddar, shredded, 2% milk, reduced fat, 8 oz, Kraft Food North America, Inc. Medium Cheddar, shredded, 2 lb Tillamook County Creamery Assoc.
Amount of crumbles	Amount of pieces that are irregular in shape of size ca. 5 mm or less	Medium Cheddar cheese, shredded, 8 oz, Lucerne Sharp Cheddar, shredded, 2% milk, reduced fat, 8 oz, Kraft Food North America, Inc. Medium Cheddar, shredded, Tillamook County Creamery Assoc.
Tactile descriptors		
Procedure: Pick and release the sample 3 times using 5 fingers and feel the oil residue on the fingers. Pick the sample one more time, squeeze it and observe how the strips stick together when you dropped them on the plate.		
Tactile oiliness	Is there oil on the fingers after pick and release?	Mozzarella cheese, low moisture, part skim milk, 2 lb, Lucerne Mild Cheddar, 2 lb, Lucerne Swiss, 2 lb, Lucerne
Adhesiveness	How much the shreds stick together after dropping them at the height of 15 cm.	Mild Cheddar, 2 lb, Lucerne Sharp Cheddar, 2 lb, Tillamook County Creamery Assoc. Parmesan fine shred, 6 oz, Lucerne

uniform length when compared with shreds from pressure-treated samples (scored 5.0 to 6.6). In addition, pressure-holding time and storage affected differences between samples, and these effects depended on pressure level. However, differences in shred length uniformity caused by pressure level, pressure time, and storage time were smaller among treated samples (1.6 units difference) compared with differences between treated and untreated samples (4.3 units difference). The length uniformity of untreated samples increased during storage, while in pressure-treated samples it decreased slightly and became stable during storage. During storage of shreds from treated cheese, changes in length uniformity differed depending on pressure treatment conditions.

Only pressure level and storage time significantly influenced changes in the amount of crumbles of the shred (Figure 1c). Moreover, the effects of pressure level and storage time were independent of each other. The panel detected a lower amount of crumbles at the higher pressure level and at longer storage times. Untreated samples had the highest amount of crumbles at d 1 (scored 7.5), then reduced to a stable level (scored ~4) after 13 d of storage. In contrast, shreds from pressure-treated samples had the lowest amount of crumbles at

d 1, with values of ~2.3 for 483 MPa and ~2.8 for 345 MPa treated cheese; the amount increased slightly at d 6 and then tended to decrease until d 27.

Adhesiveness of shreds was affected by pressure level, pressure-holding time, and storage, and the interaction effect between these factors was significant (Figure 1d). Shreds from untreated cheese tended to possess higher adhesiveness than pressure-treated cheese. Unlike other attributes, shred adhesiveness did not converge at d 27. Visual oiliness of shred also depended on all 3 factors and tended to converge after 6 d of storage (Figure 1e). At d 1, the effect of pressure level and pressure-holding time was pronounced. Shreds from untreated cheese had the lowest visual oiliness (scored 2.2), followed by those subjected to 345 MPa (scored 2.9 to 3.3), while 483 MPa-treated shreds had the highest values (scored 3.3 to 4.0). The effect of processing time was significant in 483 MPa-treated samples at d 1 with shreds from 3-min treated cheese, with lower visual oiliness (scored 2.8 to 4.0) than those from 7-min treated cheese (scored 3.3 to 3.8).

Values for tactile oiliness were significantly different among all samples (Figure 1f). The values for shreds from untreated cheese increased with storage, changing from 3.0 at d 1 to 3.8 at d 27. Regardless of

Figure 1. Sensory profiles of shredded pressure-treated and control stirred curd Cheddar cheese.

pressure level, shreds from pressure-treated cheese had lower tactile oiliness at d 6, increasing at d 13 and stabilizing at d 27. Pressure-holding time affected tactile oiliness differently in cheese treated at different pressure levels and led to different behavior during storage. Overall, samples from 345 MPa-treated cheese tended to have higher tactile oiliness than samples treated at 483 MPa regardless of storage time. At

longer pressure-holding times, higher tactile oiliness values were detected regardless of storage time.

Pressure-treated samples yielded shreds with smoother surface than those from untreated samples; however, no differences in the smoothness of the shred surface were observed at d 27 (Figure 1g). The smoothness of shred surface of untreated cheese increased during storage time, changing from 2.8 at d 1 to 5.7

Figure 2. Principal component analysis for the pressure effect on the sensory properties of stirred curd Cheddar cheese.

at d 27. On the other hand, the smoothness of shreds from treated cheese increased from 5.2 to 5.8 at d 1 to 3.9 to 6.2 at d 6 and then decreased or stabilized depending on pressure level and pressure-holding time (scored 5.7 to 6.2).

Correlation between sensory descriptors was summarized using principal component analysis, which identified 3 groups of samples (I to III). There were 2 major dimensions extracted from the data that described the samples in these groups (Figure 2). The PC1 dimension took into account 52.4% of the variance, and comprised mean length, uniformity of length, shred smooth surface, visual oiliness, and presence of crumbles. Presence of crumbles was negatively correlated with the other attributes in the shred-dimension. The PC2 dimension took into account 23.8% of the variance and it comprised tactile oiliness and adhesiveness, which were positively correlated (Fig. 2). Group I included d 1 controls characterized by high values for the amount of crumbles and the low values for visual and tactile oiliness, smooth surface, adhesiveness, shred mean length and shred length uniformity. Reflecting the ripening effect, group II included the control for d 6, 13, and 27, and was characterized by a higher shred to shred particle adhesion, smooth surface, shred mean length and uniformity, visual oiliness, and lower amount of crumbles. Also included in this group, and reflecting the effect of HPP treatments, were samples 345 MPa/3 min for

d 6, 13, and 27, 345 MPa/7 min for d 6 and 13, and 483 MPa/7 min for d 1, 13, and 27. Group III corresponded to 345 MPa/3 min for d 1, 345 MPa/7 min for d 1 and 27, 483 MPa/3 min for all sampling days, and 483 MPa/7 min for d 6. These samples were characterized by a lower shred-to-shred particle adhesion and lower tactile oiliness, but were otherwise similar to those in group II.

Texture Profile Analysis

Parameters calculated from TPA analysis for control and pressure-treated Cheddar cheese samples are shown in Table 2. During storage, the hardness of control samples decreased. The HPP treatment increased hardness at d 1 with an effect increasing with pressure level and pressurization time (Table 2). The hardness values of cheese samples pressurized 3 min at 345 and 483 MPa were not different but were significantly higher than those for untreated samples ($P < 0.05$). Similarly, samples treated 7 min at 345 or 483 MPa were not different, but had significantly higher values than untreated samples and those pressure treated for 3 min. The hardness of all pressure-treated samples decreased significantly during the remaining 60 d of storage. Samples treated for 3 min were not different from d-60 untreated samples, while samples treated for 7 min were softer or harder than the control when treated at 345 or 483 MPa, respectively. Springiness changed during the first 13 d of storage, decreasing significantly at d 60 for untreated cheese (Table 2). At d 1, HPP samples had springiness values slightly higher than untreated samples, with only 7-min treated samples significantly ($P < 0.05$) higher than control. During storage, springiness decreased for 345-MPa pressure-treated samples, reaching the lowest values at d 60, but did not for the 483-MPa treated samples. All treated samples showed higher springiness values than untreated samples, with the 483-MPa treated samples reaching the highest values. The cohesiveness of untreated samples decreased during storage, reaching the lowest values at d 60 (Table 2). All treated samples showed differences at d 1 only when compared with untreated samples. During ripening, the cohesiveness values of all pressure-treated cheese samples were lower at d 60, but were still significantly higher than untreated samples, and with no differences between pressure treatments.

Scanning Electron Microscopy

The functional and mechanical properties of cheese reflect its microstructure, which is a continuous protein matrix with fat globules dispersed in the protein

Table 2. Effect of pressure treatments and ripening time on texture profile analysis parameters.

Texture parameter	Storage (d)	Control	345 MPa		483 MPa	
			3 min	7 min	3 min	7 min
Hardness (kg)	1	13.2 ^{a,1}	15.4 ^{a,2}	16.9 ^{a,3}	15.0 ^{a,2}	17.0 ^{a,3}
	6	14.3 ^{a,1}	14.2 ^{b,1}	13.0 ^{b,2}	14.2 ^{a,1}	14.9 ^{b,1}
	13	12.3 ^{a,b,1}	10.5 ^{c,2}	11.0 ^{c,2}	17.7 ^{b,1}	12.8 ^{c,3}
	60	11.3 ^{b,1}	10.7 ^{c,1}	9.8 ^{d,2}	11.0 ^{c,1}	13.2 ^{c,3}
Springiness	1	0.75 ^{a,1}	0.77 ^{a,1,2,3}	0.81 ^{a,3}	0.77 ^{a,1,2,3}	0.79 ^{a,2,3}
	6	0.70 ^{b,c,1}	0.76 ^{a,b,2}	0.76 ^{b,2}	0.79 ^{a,b,2}	0.78 ^{a,2}
	13	0.72 ^{a,b,1}	0.75 ^{a,b,1,2}	0.75 ^{b,1,2}	0.82 ^{b,3}	0.77 ^{a,2}
	60	0.67 ^{c,1}	0.73 ^{b,2,3}	0.71 ^{c,2,3}	0.76 ^{a,3,4}	0.78 ^{a,4}
Cohesiveness	1	0.42 ^{a,1}	0.48 ^{a,1}	0.51 ^{a,2}	0.50 ^{a,2}	0.51 ^{a,2}
	6	0.44 ^{a,1}	0.48 ^{a,2}	0.48 ^{a,2}	0.50 ^{a,2}	0.50 ^{a,2}
	13	0.44 ^{a,1}	0.48 ^{a,2}	0.48 ^{a,2}	0.50 ^{a,2}	0.49 ^{a,2}
	60	0.38 ^{b,1}	0.43 ^{b,1}	0.43 ^{b,1}	0.46 ^{b,3}	0.45 ^{b,2,3}

^{a,b,c}Mean values (n = 8) in column with different superscript letters differ ($P < 0.05$).

^{1,2}Mean values (n = 8) in rows with different superscript numbers differ ($P < 0.05$).

network (Bryant et al., 1995; Rosenberg et al., 1995). The network structure determines cheese texture and is affected by composition, manufacturing process, proteolysis during ripening, and fat droplet size and distribution (Lagoueyte et al., 1994; Torres-Mora et al., 1996; Sutheerawattananonda et al., 1997). The microstructure of 1-d-old untreated and pressure-treated Cheddar cheese, as observed by scanning electron microscopy, is shown in Figure 3. The control cheese presented a porous protein network, prominent clumping, and sponge-like appearance, with fat globules of different size and shape distributed nonuniformly.

Guinee et al. (2000) reported that in Cheddar cheese fat is distributed in the casein network as discrete fat droplets or large irregular sections, which coincides with the control cheese observations reported here. The microstructure of pressure-treated cheese, however, showed a polymer network with an instant microstructure change to continuous protein matrices resembling aged Cheddar cheese. This effect was observed to a different extent depending on the pressure level and process time of the HPP treatment. Changes in microstructure were highest for 483 MPa/7 min treatment, followed by 483 MPa/3 min, 345 MPa/7 min, and 345 MPa/3 min treatments. Cheese treated at 483 MPa for 7 min had the most continuous microstructure, while the protein matrices observed in samples treated at 345 MPa for 3 and 7 min showed sections with the sponge-like appearance, while others exhibited a smooth, continuous protein network.

Changes in pH

The pH of the control cheese samples increased during storage. The initial pH increase induced by pres-

sure treatments (Figure 4a) reflected a stronger effect of pressurizing time than pressure level, with 7 min > 3 min > control. During storage, pH showed fluctuations, but at d 27, the difference in pH between HPP samples and untreated control was minimal. Messens et al. (1999) reported a pH shift of about 0.25 units 1 d after 400 MPa was applied for 6 min in Gouda cheese; they associated it with both a reduction in microbial activity to convert lactose to lactic acid and enhanced proteolysis. Casal and Gomez (1999) found a reduction in acidifying activity in lactic acid cultures after HPP treatment, even though there was no apparent loss of cell viability. On the other hand, Saldo et al. (2000a) reported about 3 log cycles reduction in starter counts after a 400-MPa treatment, which was interpreted as the cause for a higher final pH. In the work here reported, initial pH differences between controls and pressure-treated samples decreased to a minimum during storage, suggesting only minor effects of HPP treatments on global microbial and proteolytic activity.

Nonprotein Nitrogen

All pressure-treated samples showed an increase in NPN/TN (Figure 4b) and WSN/TN (Figure 4c) values during storage. Pressure level and time significantly influenced the development of NPN/TN during the 27 d of storage (Figure 4b). At d 27, control and 3-min HPP samples showed a significantly lower NPN/TN level when compared with samples treated at 385 and 483 MPa for 7 min. These results suggest that although the HPP treatments modified cheese microstructure (Figure 3), mechanical properties (Table 2), and visual and tactile sensory parameters (Figures 1 and 2), significant proteolytic activity occurred during

Figure 3. Scanning electron microscopy micrographs of pressure-treated and control stirred-curd Cheddar cheese (1000 $\times$ magnification, bar represents 10 μ m).

Figure 4. Effects of pressure treatment and ripening time on pH (a), nonprotein nitrogen (b) and water soluble nitrogen (c) during ripening of stirred-curd Cheddar cheese.

storage for both control and treated samples, with some treatments showing higher NPN/TN values. Smit et al. (1996) found a higher peptidolytic activity in pressurized starter cultures and associated it with an increase in cell lysis enhancing membrane permeability and the release of proteolytic enzymes into the cheese.

Casal and Gomez (1999) pointed out that the pressure-treated cells of some *Lactococcus* and *Lactobacillus* exhibited a greater hydrolytic activity on the C-peptide than untreated controls. These events could contribute to the release of small protein fractions and free amino acids due to microbial enzymes activity

increasing NPN values (Fox, 1988). It is also important to consider that most starter peptidases have an optimal pH higher than observed in the control cheese (Visser, 1993), and thus the increase in pH of 345 and 483 MPa treatments could reflect increased enzymatic activity. Messens et al. (1999) reported that 300 MPa/50 min pressure treatments disrupted the paracasein network, leading to a higher amount of peptides. However, in the study here reported, the experimental design covered much shorter pressure treatment times.

CONCLUSIONS

The effect of HPP treatments on the shredability of Cheddar cheese manufactured by conventional milled-curd technology (Serrano, 2003) has been shown to be similar to that reported here for Cheddar cheese manufactured by the stirred-curd approach. This observation suggests that pressure treatments could improve the early shredability of other natural cheeses of commercial interest, but this prediction should be evaluated experimentally. The use of pressure processing technology would reduce manufacturing costs of shredded cheese by eliminating cheese handling steps and reducing refrigerated storage currently required before shredding of Cheddar and other natural cheeses.

The microstructure of treated cheese samples was affected immediately by the HPP treatments, although the ripening process was not inhibited, as observed by a similar behavior of untreated and pressure-treated samples after ripening. High-pressure processing treatments of unripened cheese before shredding reduced the amount of crumbles, increased shred mean length, improved length uniformity, and enhanced surface smoothness. The visual and tactile characteristics of shreds obtained from pressure-treated stirred-curd Cheddar cheese coincided with properties deemed desirable by cheese processors (McMahon, personal communication, 2001) and showed that HPP treatment could entirely eliminate the ripening needed to get high quality shreds, yielding products of equal visual and tactile sensory attributes to those produced after 15 to 30 d of ripening.

Textural changes induced by pressure have been associated with the rupture of noncovalent interactions within the protein molecules (Messens et al., 1997), which causes protein denaturation, protein-protein dissociation, and reaggregation of proteins. High-pressure processing treatment may fuse the particles together and strengthen internal binding forces, resulting in higher cohesiveness in treated cheeses compared with untreated cheeses (Nienaber et al.,

2000). Another effect associated with pressure treatments is the change in calcium equilibrium of the calcium-caseinate complex. The same researchers reported that under high pressure, calcium-casein associations were disrupted so that calcium migrated to the aqueous soluble phase. When the pressure was released, the equilibrium was reestablished, but the association between caseins was not restored to the conditions prior to the pressure treatment. It has been reported that with storage time the differences between pressure-treated and untreated samples decrease as reflected by TPA parameters (Law et al., 1998; Saldo et al., 2000b). In the present work, the differences between pressure-treated and untreated samples, as reflected by the hardness and cohesiveness TPA parameters, became smaller with storage. At d 13, most of the differences were no longer significant. Although hardness and cohesiveness were significantly modified by pressure treatments, structural reorientation and/or additional formation of bonds during ripening of pressurized Cheddar cheese led to similar characteristics at the end of storage compared with untreated control cheese. These results showed that initial modification of mechanical properties induced by HPP appear to be associated with the changes in sensory attributes detected by panelists. This initial modification did not affect the changes in mechanical properties that occur during ripening. These observations, along with other chemical measurements made in this study during cheese ripening, suggest that HPP-treated cheese appears to ripen to a product with characteristics similar to shredded cheese currently on the market. Therefore, the shredding of unripened pressure-treated cheese appears to have minimum negative consequences on product quality.

Scanning electron microscopy analysis confirmed visually that HPP changed the microstructure of Cheddar cheeses immediately after treatment, with changes associated with pressure level and pressurizing time. The reduction in the amount of crumbles as well as the increase in desirable physical properties such as mean length, size uniformity, and surface smoothness in the HPP-treated samples suggest that when pressure is applied, proteins may be partially denatured and form a more continuous structure matrix. This protein modification was beneficial for Cheddar cheese manufactured by stirred (this study) and milled (Serrano, 2003) technology, improving sensory attributes of cheese shredded before ripening without major modifications of mechanical properties after ripening, as determined by textural profile analysis (Table 2). The literature shows some results consistent with this study. For example, the reduction of crumbli-

ness of goat cheese by high pressure has been reported by Saldo et al. (2000b). Capellas et al. (1997) reported that pressurization induced a modification in protein network in pressurized cheeses and associated these changes in protein matrices with changes in objective and subjective textural properties. Adhesiveness and tactile oiliness are both associated with the amount of oil on the surface of the cheese strips. In the study here reported, HPP-treated samples showed higher levels of these attributes after the first day, suggesting a larger amount of free fat probably caused by pressurization inducing a more continuous structure and leaving less available spaces to hold fat globules. This result could also reflect a modification in protein properties. Galazka et al. (1996) reported that high pressure reduced the emulsion properties of β -lactoglobulin due to pressure-induced protein structure modifications.

In conclusion, high-pressure treatments in terms of pressure level and pressurizing time modified the microstructure and texture properties of Cheddar cheeses. All pressure-treated cheeses immediately showed, as reflected by d 1 measurements, sensory attributes similar to those observed in untreated cheese ripened for 27 d. Consequently, the shredded cheese manufacturing industry could shred the cheese immediately after cooling if the cheeses are treated with HPP. A moderate pressure treatment of 345 MPa for 3 min was sufficient to induce the shredding characteristics of ripened cheeses, reducing storage and handling costs with expected savings of over (USD)\$15/1000 lb of cheese, considering only refrigerated storage savings (Farkas, personal communication, 2000) and not including additional savings by reducing steps in the handling of cheese blocks produced for shredding.

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