# Moderately High Hydrostatic Pressure Processing to Reduce Production Costs of Shredded Cheese: Microstructure, Texture, and Sensory Properties of Shredded Milled Curd Cheddar

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ABSTRACT: Short and moderate hydrostatic pressure (MHP) treatments accelerated the shredability of Cheddar cheese. Both MHP (345 MPa for 3 and 7 min) and higher pressure (483 MPa for 3 and 7 min) treatments applied to 1-d milled curd Cheddar cheese induced immediately a microstructure resembling that of ripened cheese. Unripened pressure-treated Cheddar cheese yielded shreds with visual and tactile sensory properties similar to those obtained from untreated 27-d-old Cheddar cheese. All pressure treatments reduced the presence of crumbles, increased mean shred particle length, improved length uniformity, and enhanced surface smoothness. Sensory evaluations showed that shredded samples of 1-d MHP-treated cheese and 27-d untreated cheese had similar sensory attributes. Pressure treatments did not affect mechanical properties of ripened cheese and milk protein proteolysis was not inhibited. These results showed that MHP would allow processors to shred milled curd Cheddar cheese immediately after block cooling with expected refrigerated storage savings of more than \$30 US/1000 kg cheese and could simplify the handling of cheese for shredding. Shreds from unripened milled curd Cheddar cheese can thus be produced with high visual acceptability and improved tactile handling using moderate levels of hydrostatic pressure.

Keywords: Cheddar cheese, moderate hydrostatic pressure, microstructure, shredability, texture

## Introduction

heddar cheese is the most widely consumed cheese in the United States of America with shredding used by cheese manufacturers to market a value-added product. Similar interest in shredded cheese has been observed elsewhere in the world. In Germany, sales of shredded cheese for pizza rose 125% between 1995 and 1996, whereas general-purpose shredded cheeses, mainly Gouda and Edam, increased by 91%. Shredding is desirable to consumers as a means to increase the surface area covered by cheese to achieve a desired culinary effects with a minimum cheese quantity (Apostolopoulos and Marshall 1994). Cheddar cheese requires about 30 d of ripening before shredding adding refrigerated storage and handling costs to the product. Key attributes improved by this ripening time are the amount of crumbles, surface smoothness, shred mean length, and uniformity of shred length. Other desirable attributes improved by ripening are shred to shred piece adhesiveness, visual perception of oiliness and residual oiliness during shred handling by consumers (McMahon 2001).

Two manufacturing technologies are commonly used in Cheddar cheese production, the traditional or milled curd and the more recently developed stirred curd approach. Milled curd involves the

MS 20040065 Submitted 2/3/04, Revised 4/8/04, Accepted 1/11/05. Authors Serrano, Velazsquez, and Torres are with Food Process Engineering Group, Dept. of Food Science & Technology, Oregon State Univ., 100 Wiegand Hall, Corvallis, OR 97331-6602. Author Serrano is with Dept. de Investigación y Posgrado en Alimentos, Facultad de Química, Univ. Autónoma de Querétaro, Centro Univ., Cerro de las Campanas, Querétaro, Mexico. Authors Velazquez and Ramirez are with Unidad Académica Multidisciplinaria Reynosa Azltán, Univ. Autónoma de Tamaulipas, Col. Aztlán, Reynosa, Tamaulipas, Mexico. Author Lopetcharat is with Unilever Research-US, Edgewater, N.J. Direct inquiries to author Torres (E-mail: <u>I Antonio. Torres@oregonstate.edu</u>). piling of Cheddar cheese blocks of about 25 cm on top of each other, allowing them to rest for about 15 min and then turning them over. This procedure (cheddaring) is repeated 2 or 3 times until the desired acid level is achieved. In the 2nd method, the drained curd is agitated continuously to reach the desired acid level. This constant agitation does not allow knitting of curd particles, but the cheese obtained has similar composition and properties to milled curd cheese and it is a faster and simpler technology to mechanize (www.milkingredients.ca).

The texture and mechanical properties of a cheese are determined by a microstructure usually described as a continuous protein matrix with a porous structure with spaces occupied by fat globules distributed in the casein network (Mistry and Anderson 1993; Lagoueyte and others 1994; Bryant and others 1995; Sutheerawattananonda and others 1997; Kheadr and others 2002). Torres-Mora and others (1996) observed that moderate pressure treatments (414 MPa for 2 min) applied to fresh curd yielded immediately a microstructure similar to aged Cheddar cheese, a finding that was later confirmed by Yang (1999). Nienaber and others (2000) subjected stirred-curd Cheddar cheese to 100 to 800 MPa (5 min, 25 °C) at different time points during the ripening process and found that treated and control cheese 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 did not differ from controls. However, the 800-MPa treated cheese had higher hardness, cohesiveness, gumminess, and chewiness when compared with controls. High pressure processing (HPP) effects on aging appear to depend on pressure conditions and cheese variety.

Saldo and others (2000b) subjected goat milk cheese at 14 °C to 50 MPa/72 h, 400 MPa/5 min, and 400 MPa/5 min followed by 50 MPa/72 h and concluded that the combination of short pressure treatments to release microbial enzymes followed by long pressure treatment to enhance their activity could accelerate cheese ripening. The possibility of accelerating Gouda cheese ripening by pressure treatments (50 to 400 MPa, 20 to 100 min) was investigated in a ripening study for up to 42 d (Messens and others 1999, 2000). Increasing the pressure and holding time led to an increase in the pH shift, defined as the difference between the pH of pressurized and untreated cheese. However, pressure treatments did not influence the soluble nitrogen and free amino acid content.

The market importance of shredded Cheddar cheese provided the justification for this work, and thus the central objective of this study was to determine the feasibility of using moderate hydrostatic pressure (MHP) (345 MPa) to reduce or eliminate the ripening needed before milled curd Cheddar cheese reaches shredability. A 2nd objective was the development of a sensory evaluation methodology for shredded cheese absent in the published literature to study the effects of pressure treatments and storage on shreds from milled curd Cheddar cheese. Also studied were the effect of pressure treatments and storage on chemical indices of ripening, cheese microstructure, and mechanical properties.

# **Materials and Methods**

# **Cheese samples**

Fresh cow's milk milled Cheddar cheese blocks (18.2 kg) provided by Tillamook County Creamery Association (Tillamook, Oreg., U.S.A.) were collected immediately after cooling and transported refrigerated for moderate high pressure processing at the facilities of Avure Technologies, Inc. (Kent, Wash., U.S.A.). Blocks were cut into small blocks ( $7 \times 3.5 \times 18$  cm) and vacuum-packed for treatments within 24 h of sample collection at 345 and 483 MPa for 3 and 7 min. Untreated and pressure-treated cheeses were placed in storage for aging at 7 °C for further analysis.

# **Cheese composition**

Cheese samples were analyzed in triplicate after 1, 6, 13, and 27 d of ripening for moisture, protein, fat, salt, and pH (Marshall 1992). Proteolysis throughout storage was monitored using the micro-Kjeldahl method (AOAC 2000) to measure total nitrogen (TN), water-soluble nitrogen at pH 4.6 (WSN), and non-protein nitrogen (NPN) measured after removing proteins with a 12% trichloroacetic acid (TCA) solution.

# **Texture analysis**

Cubes of 20 mm cut using a custom-built wire cutter to obtain cubes of uniform shape and size were kept in sealed plastic bags to avoid surface drying and held at 4 °C for 12 h before compression tests performed using a TA.XT2 Texture Analyzer (Stable Micro Systems, Vienna Court, U.K.). Texture profile analysis (TPA) at 50% was performed at 1 mm/s of crosshead speed with a 25-kg load cell and 50-mm-dia 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 cheese pieces (about 3-mm cubes) 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 labeled biopsy bags (Fisher Scientific 15-182-506H, Hampton, N.H., U.S.A.), 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 labeled 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, Mass., U.S.A.).

# Sensory analysis

A descriptive analysis (Lawless and Heymann 1998; Meilgaard and others 1999) was performed by a trained descriptive panel consisting of 9 members (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 handevaluated texture terms (tactile appearance) to characterize shredded cheese. Panelists were presented with an array of commercial shredded cheese samples to aid in developing these terms. Descriptors selected for appearance were visual oiliness, surface smoothness, shred mean length, uniformity of shred length, and amount of crumbles. Tactile perception terms selected by the panel were shred adhesiveness and tactile oiliness defined as oily residues on fingers after shred handling (Table 1). Panelists entered numeric responses using a 9-point intensity rating scale anchored with "none" 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 1st 2 sessions, the panel determined the terminology and established definitions for the 7 descriptors; in the following 2 sessions, judges suggested standards and also scored them; and the final 2 meetings were used for practice sessions with shredded samples of commercial and pressure-treated cheese. For sensory analysis of duplicate experimental samples, panelists evaluated 10 samples per evaluating session.

Cheeses were shredded using a food processor (Model A200, Hobart Corp., Troy, Ohio, U.S.A.) with a front-mounted taper attachment hub (model VS9) and adjustable shredder plate (3/16 inch, model VS9). Five grams of shredded cheese were presented in 3-digit random-coded plastic sample cups (50 mL) covered with lids to avoid surface drying and held at constant temperature (4 °C) for at least 2 h before sensory analysis. Panelists poured the cup into 15.2-cm-dia 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 the pressure treatment. All panelists were monetarily compensated for their participation in the study.

# Statistical analysis

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

Definition	Cheese standards				
Appearance desc	riptors				
30 cm from the sample and evaluate without conside	ering the presence of crumbles and fines				
How shiny the sample is	Parmesan fine shred,ª 0.17 kg Sharp Cheddar, <sup>b</sup> 0.9 kg Swiss,ª 0.9 kg				
How smooth the strip is outside	Sharp Cheddar, <sup>b</sup> 0.9 kg Mild Cheddar, <sup>a</sup> 0.9 kg Mozzarella, low moisture, part skim milk, <sup>a</sup> 0.9 kg				
Average length of cheese shreds	Yellow paper shreds: 0.01, 0.02, and 0.04 m				
How uniform in length the strips are, not considering presence of crumbles and fines	Medium Cheddar, <sup>a</sup> shredded, 0.23 kg Sharp Cheddar, shredded, 2% milk, reduced fat, <sup>c</sup> 0.23 kg Medium Cheddar, <sup>b</sup> shredded, 0.9 kg				
Amount of pieces that are irregular in shape of size about 0.005 m or less	Medium Cheddar, <sup>a</sup> shredded, 0.23 kg Sharp Cheddar, <sup>c</sup> shredded, 2% milk, reduced fat, 0.23 kg Medium Cheddar, <sup>b</sup> shredded, 0.23 kg				
Tactile descrip	tors				
ease the sample 3 times using 5 fingers and feel the how the strips stick together when you dropped the	e oil residue on the fingers. Pick the sample 1 more time, m on the plate.				
Is there oil on the fingers after pick and release?	Mozzarella,ª low moisture, part skim milk, 0.9 kg Mild Cheddar,ª 0.9 kg Swiss,ª 0.9 kg				
How much the shreds stick together after dropping them from a height of 15 cm	Mild Cheddar, <sup>a</sup> 0.9 kg Sharp Cheddar, <sup>b</sup> 0.9 kg Parmesan fine shred, <sup>a</sup> 0.17 kg				
	Definition   30 cm from the sample and evaluate without considered   How shiny the sample is   How smooth the strip is outside   Average length of cheese shreds   How uniform in length the strips are, not considering presence of crumbles and fines   Amount of pieces that are irregular in shape of size about 0.005 m or less   Tactile descrip   ease the sample 3 times using 5 fingers and feel the a how the strips stick together when you dropped the   Is there oil on the fingers after pick and release?   How much the shreds stick together after dropping them from a height of 15 cm				

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

cerne Foods Ltd

<sup>b</sup>Tillamook County Creamery Assoc. <sup>c</sup>Kraft Food North America, Inc.

## **Results and Discussion**

#### Table 2-Milled curd Cheddar cheese composition at different storage time

Time (d)

## Composition

Chemical composition was determined at 1, 13, and 27 d. As expected, the moisture, fat, protein, and sodium contents were not significantly different among the pressure treatments and storage time (Table 2). No differences among pressure treatments and storage time samples were observed, and thus only average values were reported. Similar results were obtained by Capellas and others (2001) who reported that pressure treatments (100 to 400 MPa) 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 pressure-treated and untreated cheese are summarized in Figure 1. The samples were significantly different in all attributes contributing to overall differences detected by MANOVA. The mean length of the control samples at day 1 (4.4) was significantly shorter than those for pressuretreated samples. The differences in mean length between samples at the different pressure level and pressure-holding time were much smaller than differences with respect to the control (Figure 1a). During storage time, the processing time effect was dominant and lowered differences between all samples and the control. Pressurizing cheese before shredding significantly improved the length of shreds, giving values on day 1 (5.7 to 6.9) similar to or longer than the length of shreds from control cheese stored for 27 d (6.1).

On day 1, the shreds of pressure-treated samples were all more uniform than those of control cheese (2.8) (Figure 1b) and the panel rated samples pressure-treated at 483 MPa for 7 min the most uniform shreds (6.9). Shred uniformity of control cheese reached its peak after 27 d of storage (6.19); however, equivalent values were

1 13 27 Moisture (%)  $28.4 \pm 0.4$ 28.1 ± 2.2  $34.9 \pm 0.6$ Fat (%)  $35.2 \pm 0.7$  $35.5 \pm 0.4$ 35.1 ± 0.7 Protein (%)  $32.8 \pm 1.2$  $33.7 \pm 1.5$ 30.8 + 1.2Salt (mg/kg cheese)  $193.5 \pm 2.0$  $197.5 \pm 5.6$  $200.3 \pm 2.6$ 

achieved on day 1 through treatments at 345 MPa for 7 min (6.2), 483 MPa for 3 min (6.4), or 483 MPa for 7 min (6.9). The behavior of the uniformity of length of the shred pressurized at different conditions depended on pressure level, processing time, and storage; however, most of the samples possessed at least equivalent uniformity as the control on day 13 and day 27.

The presence of crumbles depended on pressure levels and processing times; however, storage time independently influenced the amount of crumbles for shreds obtained from pressure-treated cheese allowing the comparison of experimental means (Figure 1c). On day 1 and day 6, shreds from control cheese had higher amount of crumbles (5.9 and 3.6, respectively) than those obtained from treated cheese (1.8 to 2.8 and 2.2 to 2.3, respectively). Cheese treated at 483 MPa tended to have less crumbles (about 2.2) than those treated at 345 MPa (2.3), but the difference was small (0.1). Processing time was important at 345 MPa with cheese held at this pressure for 3 min (2.6) having more crumbles than those treated for 7 min (2.3); however, both were lower than the control (3.6). The storage time effect was more pronounced on the control as compared with pressure-treated cheeses. The presence of crumbles in the control samples decreased when storage time increased from 5.9 on day 1 to 1.8 on day 27. Apparently,

control samples on day 27 yielded shreds with less crumbles than cheese treated at 345 MPa for 3 min (2.4 to 2.8) for all storage days.

On average, higher pressure levels yielded more adhesive shreds regardless of other conditions and shred adhesiveness increased with storage time (Figure 1d). On day 1, the pressure treatment effect was dominant and significant (shred adhesiveness ranged from 1.6 to 3.4) and increased with storage time until day 27, when there was no difference detected. Only pressure level and storage time significantly changed the visual oiliness of cheese shreds. On day 1, pressure treatments increased shred visual oiliness with subsequent values decreasing over time, that is, from 3.7 to 3.2 and from 4.8 to 3.3 in samples treated at 345 MPa and 483 MPa, respectively. Values increased over time (2.6 to 3.1) in shred samples from untreated cheese (Figure 1e). Unlike visual oiliness, only storage time caused changes in tactile oiliness between samples (Figure 1f). The tactile oiliness was detected highest on day 1 (4.1) and lowest on day 6 (3.3), regardless of treatment. The tactile oiliness level seemed to be stable after day 6, with gradual fluctuation for the control samples and less fluctuation in treated samples; however, there was no significant difference detected between day 13 and day 27. Analysis of variance revealed that only storage time significantly influenced the smoothness of shred surface. However, the shred surface of the control at day 1 was the least smooth among the samples (Figure 1g). Pressurization at 345 and 483 MPa regardless of processing time caused the shred surface to be as smooth as untreated cheese stored at least 6 d.



Correlation between sensory descriptors was summarized using principle component analysis, which identified 3 groups of samples. There were 2 major dimensions extracted from the data, shred-dimension and oily-dimension (Figure 2). Shred-dimension (principal component 1,PC1) took into account 59% of the variance and it comprised mean length, uniformity of length, shred smooth surface, shred adhesiveness, and presence of crumbles. The presence of crumbles was negatively correlated with the other attributes in the shred-dimension. Oily-dimension (principal component 2, PC2) (20.3%) comprised tactile oiliness and visual oiliness, which were positively correlated (Figure 2). The PCA plots show that control samples were characterized by low-quality shred characteristics (high amount of crumbles, short shreds, nonuniform shreds, and sticky shred), and as expected, shred quality of the control samples improved as storage time increased. Pressure treatments improved shred quality as seen in PCA plot with most of the samples clustered on the right side of the plot, which indicates high-quality shreds (Figure 2). However, pressurization caused shred to be oily, as seen in the samples pressurized at 483 MPa for 3 min, but this characteristic diminished after 6 d of storage. The PCA plot also shows that pressurizing Cheddar cheese at 345 MPa and stored for 1 d or at 483 MPa and stored for 3 d yielded an equivalent shred quality to untreated cheese stored for 27 d. These results favor the lowest cost alternative, that is, processing milled Cheddar cheese at the lowest pressure level and shortest time yielded high-quality shreds immediately after the pressure treatment.

# Texture profile analysis

Parameters calculated from TPA analysis for the control and pressure-treated Cheddar cheese samples are shown in Table 3. During storage, the hardness of control samples decreased after 60 d of storage. On day 1, all pressure-treated samples were softer than controls, but there were no significant differences in hardness (P <0.05) caused by pressure level or pressurization time. During storage time, the hardness of all pressure-treated samples fluctuated during the 1st 13 d and decreased on day 60 as in the untreated samples but to significantly lower values. For untreated cheese samples, springiness did not change during the 1st 13 d of storage, decreasing only on day 60. Springiness on day 1 showed just a small decrease only for the 3 min-345 MPa and 7 min-483 MPa treatments with respect to untreated samples. This parameter did not decrease during storage for pressure-treated samples. Springiness values at day 60 were not different among pressure treatments but were significantly higher to values for untreated samples stored for the same time. The cohesiveness of untreated samples decreased only for the sample stored for 60 d. Treated samples showed at day 1 no differences when compared with untreated samples. The cohesiveness for all pressure-treated cheese samples on day 60 was lower than on day 1, but significantly higher than for untreated samples.

# Scanning electron microscopy

The microstructure of 1-d-old untreated and pressure-treated Cheddar cheese, as observed by SEM, is shown in Figure 3. The



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Table 3_	Effects of	nressure tr	eatments and	l rinening	ı time on	texture	nrofile	analysis		narameters
iable 3-	Ellects of	pressure u	eauments and	i ripening	i ume on	lexture	prome	anaiysis	I FAJ	parameters.

	Storage (d)	Control	345	MPa	483 MPa		
Texture parameter			3 min	7 min	3 min	7 min	
Hardness (kg)	1	16.5 a,1	15.0 a,2	14.1 a,2	14.9 a,2	14.1 a,2	
	6	15.9 a,1	17.0 b,1	14.6 a,2	14.2 a,b,2,3	13.2 a,b,3	
	13	13.6 b,1,2,3	14.7 a,1	14.1 a,1,2	13.3 b,2,3	12.8 b,3	
	60	13.5 b,1	12.0 c,2	11.4 b,2,3	11.6 c,2,3	10.9 c,3	
Springiness	1	0.76 a,b,1	0.72 a,2	0.74 a,b,1,2	0.74 a,b,1,2	0.73 a,2	
	6	0.77 a,1	0.74 a,1,2	0.77 a,1,2	0.75 a,1,2	0.74 a,2	
	13	0.74 b,1	0.74 a,1	0.72 b,1	0.73 a,b,1	0.75 a,1	
	60	0.66 c,1	0.73 a,2	0.73 a,b,2	0.71 b,2	0.72 a,2	
Cohesiveness	1	0.46 a,1	0.46 a,1	0.48 a,1	0.46 a,1	0.46 a,b,1	
	6	0.47 a,1	0.46 a,1	0.47 a,1	0.46 a,1	0.46 a,b,1	
	13	0.44 a,1	0.45 a,b,1,2	0.46 a,b,1,2	0.45 a,1,2	0.47 a,2	
	60	0.36 b,1	0.44 b,2,3	0.45 b,3	0.42 b,2	0.44 b,2,3	

<sup>a</sup>Mean values (n = 8) in column followed by different letters (a, b, c) differ (P < 0.05). Mean values (n = 8) in rows followed by different numbers (1, 2, 3) differ (P < 0.05).

control cheese presented a porous protein network, prominent clumping, and sponge-like appearance with fat globules of different size and shape distributed nonuniformly.

The network structure determines cheese texture and is affected by composition, manufacturing process, proteolysis during ripening, and fat droplet size and distribution (Lagoueyte and others 1994; Torres-Mora and others 1996; Sutheerawattananonda and others 1997). Guinee and others (2000) reported that in Cheddar cheese, fat is distributed in the casein network as discrete fat droplets or large irregular sections, which coincide with the observations here reported. The microstructure of pressure-treated cheese, however, showed a polymer network with an instant microstructure change to a continuous protein matrices resembling aged Cheddar cheese (Torres-Mora and others 1996). This effect was observed to different extents, depending on the pressure level and process time of the pressure treatment. Changes in microstructure were highest for 483 MPa for 7 min treatment followed by 483 MPa for 3 min, 345 MPa for 7 min, and 345 MPa for 3 min. Cheese treated at 483 MPa for 7 min had the most continuous microstructure, whereas the protein matrices observed in samples treated at 345 MPa for 3 and 7 min showed sections with the sponge-like appearance and others with a smooth continuous protein network.

## Changes in pH

The pH of the control cheese samples increased during storage (Figure 4a). Pressure treatments increased initial pH with changes reflecting a stronger pressure level than pressure-holding time. During storage, pH fluctuated and the pH difference on day 27 was lower than initial pH difference (day 0) but remained higher for pressure-treated samples with 483 MPa > 345 MPa > control. A pH shift of about 0.25 units 1 d after pressure treatment was reported by Messens and others (1999) in Gouda cheese subjected to 400 MPa for 6 min, and they associated this effect with a reduction in microbial activity to convert lactose to lactic acid and with an enhancing in proteolysis responsible for pH increases. Casal and Gomez (1999) found a reduction in acidifying activity in lactic acid cultures after pressure treatment even though there was no apparent loss of cell viability. Saldo and others (2000a) reported a reduction in starter counts by about 3 log cycles as a result of a 400 MPa treatment causing a higher final pH. The results obtained in the present work indicated that initial pH differences between untreated and MHP-treated samples decreased during storage, suggesting minor changes in global microbial and proteolytic activity caused by pressure treatments.

## Non-protein and water soluble nitrogen

Pressure level and pressurization time significantly influenced the development of non-protein nitrogen/TN (NPN/TN) during the 27 d of storage (Figure 4b) but retaining all significant levels of proteolytic activity. On day 1, untreated and MHP-treated samples showed similar levels in NPN/TN and WSN/TN. The control and all MHP-treated samples showed NPN/TN values increasing during storage, suggesting retention of proteolytic activity. These results suggest that although pressure treatments modified protein structure (Figure 3), sensory parameters (Figure 1), and mechanical properties (Table 3), milk protein remain susceptible to proteolysis and explained the significant proteolytic activity detected during storage for all samples. WSN/TN increased on day 6 for all cheese samples, including controls, and showed minor changes on day 13 and day 27. With the exception of the 483 MPa/3 min treatment, significant effects of pressure level and pressuring time were not detected during storage.

Smit and others (1996) found a higher peptidolytic activity in pressure-treated starter cultures, and they associated this observation to an increase in cell lysis enhancing membrane permeability and releasing additional proteolytic enzymes into the cheese. Casal and Gomez (1999) pointed out that the cells of some Lactococcus and Lactobacillus subjected to pressure treatments exhibited a greater hydrolytic activity on the C-peptide when compared with untreated cells. These events could contribute to the release of small protein fractions and free amino acids due to microbial enzyme activities increasing NPN values (Fox 1988). Also important to consider is that most starter peptidases have an optimal pH higher than the one observed in the control cheese (Visser 1993), and thus the increase in pH of 345 and 483 MPa treatments could have increased enzymatic activity. Messens and others (1999) reported that 300 MPa/50 min pressure treatments disrupted the paracasein network leading to a higher amount of peptides. However, it should be noted that much shorter processing times were used in the study here reported.

The results showed conclusively that pressure treatments improved the visual and tactile sensory properties of shredded Cheddar cheese. By reducing the amount of crumbles, increasing mean length, improving length uniformity, and enhancing surface smoothness, it was possible to obtain shreds from unripened cheese with a higher visual acceptability and improved tactile handling. The results obtained from the TPA analysis confirmed that the microstructure of treated cheese samples was affected immediately by the pressure treatments, although the ripening process was not inhibited as observed by a similar behavior of untreated and treated samples on day 60 of storage.

Texture changes induced by pressure have been associated to rupture of noncovalent interactions within the protein molecules (Messens and others 1997). The rupture of noncovalent interactions causes protein denaturation, protein-protein dissociation, and re-aggregation of proteins. Pressure treatments may fuse the particles together and strengthen internal binding forces resulting in higher cohesiveness in treated cheeses compared with untreated cheeses (Nienaber and others 2000). Other effects 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, and as a result, calcium migrated to the aqueous soluble phase. When pressure was released, the equilibrium was reestablished, but the association between caseins was not restored to conditions before the pressure treatment.

It has been reported that during storage, the texture differences between pressure-treated and untreated samples become smaller as reflected by TPA parameters (Law and others 1998; Saldo and others 2000b). Similar observations were made in this study; the differences between pressure-treated and untreated samples as reflected by the hardness and cohesiveness TPA parameters became smaller with storage. On day 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 as compared with the untreated control cheese. These results showed that initial modification of mechanical properties induced by pressure 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.

The SEM analysis showed visually major changes in the microstructure of Cheddar cheese immediately after treatment, with changes associated with pressure level and pressurizing time. The reductions in the amount of crumbles as well as increases in desirable physical properties such as surface smoothness and shred



copy micrographs of pressure-treated and control milled cheese (1000× magnification, bar represents 10 μm)



Figure 4 – Effects of pressure treatment and ripening time on pH (a), non-protein nitrogen (b), and water-soluble nitrogen (c) during ripening of milled curd Cheddar cheese

mean length and uniformity in treated samples suggest that when pressure is applied, proteins may be partially denatured and form a more continuous structure matrix. There is some evidence in the literature showing consistent results with this study. For example, the reduction of crumbliness of goat cheese by high pressure has been reported by Saldo and others (2000b). Capellas and others (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 oiliness are associated with the presence of oil on the cheese surface. In the study here reported, pressure-treated samples showed a higher level of these attributes since the 1st day, suggesting a larger amount of free fat probably caused by pressurization when it induced a more continuous structure, leaving less available spaces to hold fat globules. However, it could reflect also a modification in protein properties as reported by Galazka and others (1996) who found that high pressure treatments reduced the emulsion properties of β-lactoglobulin due to pressure-induced protein structure modifications.

In conclusion, moderate pressure treatments in terms of pressure level and pressurizing time modified immediately the microstructure and texture properties of Cheddar cheese. Pressure-treated cheeses showed immediately, as reflected by day 1 measurements, the same sensory attributes observed in untreated cheese ripened for 27 d. Consequently, the shredded cheese manufacturing industry could shred stirred curd Cheddar cheese immediately after cooling by treating cheeses with MHP. A treatment of 345 MPa for 3 min was sufficient to induce the shredding characteristics of ripened cheeses, reducing storage and handling costs with expected refrigerated storage savings of more than \$30 US/ 1000 kg cheese (Farkas 2000) in addition to cost reductions made possible from fewer steps in handling cheese blocks for shredding.

These modifications were associated with a modification of cheese microstructure without inhibiting the proteolytic activity needed for cheese ripening during storage. The effectiveness of pressure treatments on the shredability of Cheddar cheese manufactured by the stirred curd technology has been shown to be similar to the results here reported (Serrano 2003). This is an encouraging observation suggesting that pressure treatments could improve the early shredability of other natural cheeses of commercial interest.

## Conclusions

The behavior difference in shreds obtained from pressure-treated and unripened control cheese showed that processors could use pressure to eliminate entirely the ripening needed to get high-quality shreds and still obtain products with equal sensory attributes to those produced from Cheddar cheese ripened for about 30 d. Shreds from unripened milled curd Cheddar cheese can thus be produced with high visual acceptability and improved tactile handling using moderate levels of hydrostatic pressure. This MHP treatment would reduce manufacturing costs by eliminating several steps in the handling cheese of cheese blocks and the refrigerated storage currently required to ripen Cheddar cheese before shredding.

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