

# Yield, composition and rheological characteristics of cheddar cheese made with high pressure processed milk

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## Abstract

High Pressure (HP) treatment of milk prior to cheese-making was shown to increase the yield of cheese due to increased protein and moisture retention in cheese. Cheeses were made with raw milk or milk treated with high temperature short-time (HTST) pasteurization, and HP treatments at two levels (483 and 676 MPa) at 10 °C, 483 MPa HP at 30 °C, and 483 MPa HP at 40 °C. Cheese yield, total solids, protein, fat and salt contents were evaluated, and fat and protein recovery indices were calculated. Cheeses from HP treatments of 676 MPa at 10 °C and 483 MPa at 30 °C exhibited wet yields of 11.40% and 11.54%, respectively. Protein recovery was 79.9% for HP treatment of 676 MPa at 10 °C. The use of slightly higher pressurization temperatures increased moisture retention in cheese. Viscoelasticity of cheeses was determined by dynamic oscillatory testing and a creep-recovery test. Rheological parameters such as loss ( $G''$ ) and storage ( $G'$ ) moduli were dependent on oscillation frequency. At high (173 rad/s) and low (2.75 rad/s) angular frequencies, cheeses made from milk treated at 483 MPa at 10 °C behaved more solid-like than other treatments. Creep tests indicated that cheeses from milk treated with 483 MPa HP at 10 °C showed the smallest instantaneous compliance ( $J_0$ ), confirming the more solid-like behavior of cheese from the 483 MPa at 10 °C treatment compared to the behavior of cheeses from other treatments. Cheeses made with pasteurized milk were more deformable, exhibited less solid-like behavior than cheeses made with HP treated milk, as shown by the  $J_0$  value. With more research into bacteriological implications, HP treatment of raw milk can augment Cheddar cheese yield with better curd formation properties.

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**Keywords:** High pressure; Milk; Cheddar cheese; Rheology

## 1. Introduction

Cheese and other dairy products are important components in the American diet. Average cheese consumption in the US increased from 3.50 kg per person in the 1970s to 13.87 kg per person in 2003, an increase of 296% (IDFA, 2004). Among the different varieties of cheeses produced, Cheddar cheese is second largest, accounting for 32.10% of total cheese production in the US. In 2003, total cheese production in the US was 3.90 billion kg, of which 1.25 billion kg were of Cheddar cheese (IDFA, 2004).

High Pressure (HP) processing is a nonthermal technology that is slowly being adopted by the food industry. Unlike thermal treatments, HP does not promote the degradation of nutrients, flavors or color of foods (Hoover, 1993; Pothakamury, Barbosa-Cánovas, Swanson, & Meyer, 1995). On the other hand, HP treatments have the ability to modify the structure of certain food macrocomponents such as proteins, mainly by disruption of noncovalent interactions (Huppertz, Kelly, & Fox, 2002; Needs, Stenning, Gill, Ferragut, & Rich, 2000). The potential applications of HP in the dairy industry (Datta & Deeth, 1999) and the effects of HP on the constituents of milk (Huppertz et al., 2002) were recently reviewed, and authors suggest potential benefits to the dairy industry by HP technology.

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HP treatment of milk causes casein micelle disruption and denaturation of the whey protein  $\beta$ -lactoglobulin ( $\beta$ -lg) at pressures above 100 MPa and, to a lesser degree  $\alpha$ -lactalbumin ( $\alpha$ -la) at pressures above 300 MPa (Gaucheron et al., 1997; López-Fandiño, Carrascosa, & Olano, 1996; López-Fandiño & Olano, 1998). Needs et al. (2000) reported that whey from cheese milk samples treated at 600 MPa contained 30% less protein than control samples because whey proteins become incorporated into the curd during cheese manufacturing. It was suggested that the application of HP to milk for cheese production could lead to increased cheese yield and modification of cheese texture and composition (Needs et al., 2000). López-Fandiño et al. (1996) reported that pressurization of milk for 30 min at 300 and 400 MPa caused an estimated mean curd weight increase of 14% and 20%, respectively, and a substantial decrease in protein loss in the whey fraction (7.5–15%, respectively).

The investment in a 600 MPa HHP food processor is of the order of 0.7–3.5 million dollars. Processing 6000 l/h at 600 MPa without holding time costs around 5–6 cents per litre (Ledward et al., 1995). The combination of pressure and temperature for each specific product will define the most cost effective production system. An increase of even 0.5% and up implies significant profits for the cheese manufacturing industry. On a large scale, the benefits brought about by a small increase in cheese yield may make the industrial application of HP technology a feasible option, as long as sensory and quality characteristics of cheese are not compromised.

The objectives of the present research were to determine the effect that HP processing of raw milk, as compared to pasteurized milk, had on the yield, composition and rheology of Cheddar cheese, understand the effect of treatment temperature at selected pressure on yield, and to rheologically characterize the visco-elastic components of Cheddar cheeses made with HP processed milk.

## 2. Materials and methods

### 2.1. Milk processing

Two different batches of fresh raw and high temperature short-time (HTST) (72 °C, 15 s) milk samples were obtained from the Washington State University Creamery. Fat and protein contents were determined by Babcock and dye-binding methods (Marshall, 1992). Whole raw milk was adjusted to a butterfat to protein (F:P) ratio of 1.2. HP treatment was applied at two levels: 483 and 676 MPa at 10 °C, and at the lower pressure (483 MPa) at 30 and 40 °C. HP treatment was applied in a warm isostatic press system (Engineered Pressure Systems, Inc., Andover, MA, USA) with a cylindrical pressure chamber (0.25 m height, 0.10 m diameter). A 5% Mobil Hydrasol 78 water solution was used as the pressurizing medium. Milk was treated in the batch mode by pouring 1.7 l of standardized milk (10 °C) into a plastic bag. The bag was heat sealed, placed in the

pressure vessel, the chamber closed and the HP treatment applied for a holding time of 5 min. The rate of pressure increase was approximately 2.4 MPa/s. The time required to reach the pressure treatment (come-up time) was 3.4 min for 483 MPa and 4.6 min for 676 MPa. The come-down time was approximately 12 s. For the treatments at 30 and 40 °C, the pressure vessel was previously heated to the desired temperature and each 1.7 l batch of milk was also brought to the treatment temperature prior to HP treatment.

### 2.2. Cheese-making

Cheeses were prepared in duplicate by the method described by Kosikowski (1977) with slight modifications, as follows. Ten kilograms of milk were placed in a 10 l cheese vat built at Washington State University, and temperature was adjusted to 31.1 °C. The milk was inoculated with 0.02% of DVS-603 lactic starter (mixture of *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*), provided by Chr. Hansen Inc., (Milwaukee, WI, USA) and allowed to incubate in the cheese vat at 31.1–32.2 °C for 30 min. After 26 min during coagulation, 2 ml of calcium chloride solution (Cal-Sol, Chr Hansen Inc., Milwaukee, WI, USA) was added for a 0.02% concentration of CaCl<sub>2</sub> in cheese milk. After 30 min from the initiation of coagulation, chymosin (Chy-Max, Chr Hansen Inc., Milwaukee, WI, USA) was added at a rate of 0.099 ml/kg of milk. Each 1 ml of enzyme was diluted in 40 ml of tap water prior to addition. Curd strength was tested by immersion of a spatula into the curd after 15 min from chymosin addition and monitored every minute. Once a clean cut was obtained (16–30 min after enzyme addition) the curd was cut into 0.5 cm cubes using wire cheese knives, and cheese cubes were allowed to heal for 5 min. The temperature of the curd was gradually increased from 31.1 to 37.8 °C over a 30 min period with steady manual agitation. The curd cubes were cooked at 37.8 °C for 45 min with 1-min of agitation every 5 min. After 45 min,  $\frac{1}{3}$  of the whey was drained. When the whey level was approximately 2.5 cm above the curd, draining was stopped and curd cubes were allowed to mat for 5 min. At this point, the jacket of the vat was filled with water at 38 °C and the remaining whey was drained. The curd mat was cut longitudinally to allow for whey draining. Whey samples were obtained and titratable acidity was determined every 15 min according to standard procedures (Marshall, 1992). After 15 min the curd columns were cut into six blocks of 10 × 10 × 10 cm, distributed along the cheese vat and turned over every 15 min. After 45 min of whey drainage individual blocks were piled into two-piece blocks. These double blocks were turned over every 15 min, exposing new sides each time, until the end of the cheddaring process, i.e. when titratable acidity of whey was between 0.53% and 0.56% lactic acid. The curd blocks were placed on a tray, cut into 1 × 1 × 1 cm cubes and returned to the vat for salting. Coarse salt was added in three equal portions,

5 min apart, at a rate of 2.3 kg salt/100 kg cheese. The salt was allowed to dissolve prior to addition of the next portion. The cheese cubes were pressed in a Wheeler Cheese Press (Wheeler Engineering Dunchideock Exeter, UK) at 2.75 N/cm<sup>2</sup> for 18 h. Each 1 kg cheese was vacuum-packed (3-mil high barrier nylon/EVOH/PE vacuum pouches; Vacuum = -27" Hg; model: 250 Ultravac; KOCH, Kansas City, MO, USA) and stored at 1.7 °C. Cheeses were periodically turned over to allow for the development of a uniform structure. Wet yield was calculated immediately after removal from the press. After 65 days of aging, cheeses were evaluated for fat, protein, salt, total solids and rheological properties.

### 2.3. Proximate analysis of cheese

Moisture, protein, fat, and salt analyses of the experimental cheeses were conducted using standard procedures (Marshall, 1992). Moisture content of cheese was determined by placing approximately 5 g of finely grated cheese into a previously dried aluminum tray. The samples were allowed to dry for 18 h in a forced-air oven (Stabil-Therm, Blue M, Williamsport, PA, USA) at 100 °C. Moisture was reported as grams of water per hundred grams of wet sample. Protein content was determined by the dye-binding method using a Udy Colorimeter (UDY Corp., Fort Collins, CO, USA) and appropriate reagents were used to determine the protein content. Fat content of the cheeses was determined by the Babcock method (Marshall, 1992). Salt content in cheese was determined by standard methods (Marshall, 1992) using a chloride analyzer (model 926, Corning Glass Works, Medfield, MA, USA).

### 2.4. Cheese yield

Wet cheese yield was determined immediately after pressing as the amount of cheese obtained per 100 kg of cheese milk. The adjusted yield (Kosikowski, 1977) was calculated for a cheese with 37% moisture and 1.5% salt as

Adjusted yield

$$= \text{Actual yield} * \frac{100 - (\% \text{Actual moisture} + \text{salt})}{100 - (\% \text{Desired moisture} + \text{salt})}$$

Protein and fat recoveries were calculated as

Component recovery %

$$= \frac{\% \text{Component in product} * \text{quantity of product}}{\% \text{Component in milk} * \text{quantity of milk}} * 100.$$

### 2.5. Visco-elastic measurements

Rheological characterization of Cheddar cheeses made with HP treated milk was done by dynamic oscillatory and creep-recovery tests according to the methods explained by Steffe (1996). Both tests were performed using a UM/MC 120 Rheometer (Paar Physica USA Inc., Glen Allen, VA,

USA) and a texturized 25 mm dia parallel plate geometry (Paar Physica USA Inc., Glen Allen, VA, USA) with 1 mm gap. Cheeses were sliced into 25 mm diameter and 1 mm thick disks with a cheese slicer and wrapped with plastic film to prevent dehydration. Cheese temperature was controlled at 20 ± 0.1 °C using a circulating water bath. Six subsamples from each treatment were measured. The linear visco-elastic region was determined through an amplitude sweep test within 370–7250 Pa and angular frequency of 62.8 s<sup>-1</sup>. The dependence of storage (*G'*) and loss (*G''*) moduli on frequency were evaluated through a frequency sweep (0.1–300 s<sup>-1</sup>) set at 1000 Pa. Creep tests were performed by subjecting the cheese sample to a constant shear stress of 1000 Pa and compliance was monitored for 120 s. After that, the stress was removed and samples were allowed to recover for 720 s. Creep and recovery data were analysed using US200 software (Paar Physica USA Inc., Glen Allen, VA, USA). Compliance (strain/stress) data during creep phase were fitted by the following Burgers model (Physica US 200 Manual, 1993; Steffe, 1996):

$$J(t) = J_o + J_m \left( 1 - \exp\left(\frac{-t}{\lambda}\right) \right) + \frac{t}{\epsilon_o},$$

whereas the recovery phase was fitted by

$$J(t) = J_{\max} - J_o - J_m \left( 1 - \exp\left(\frac{-t}{\lambda}\right) \right),$$

where *J(t)* is the creep compliance, *J<sub>o</sub>* is the instantaneous compliance, *J<sub>m</sub>* is the visco-elastic compliance, *λ* is the mean retardation time, *ε<sub>o</sub>* is the zero shear viscosity and *J<sub>max</sub>* is the maximum creep compliance. Other parameters obtained were the elastic share of compliance (*J<sub>e</sub>/J<sub>max</sub>*) and the viscous share of compliance (*J<sub>v</sub>/J<sub>max</sub>*) to determine the contribution of the elastic and viscous components to total visco-elasticity of Cheddar cheeses.

### 2.6. Statistical analysis

The experimental design was a completely randomized design with six treatments by triplicate: raw milk, HTST pasteurized milk, HP processing at 676 MPa and 10 °C, HP at 483 MPa and 10 °C, HP at 483 MPa and 30 °C, and at 483 MPa and 40 °C. Data were analysed by the general linear model using SAS software (SAS, 1990) and significant differences were established at *α* = 0.05 by Tukey's test.

## 3. Results and discussion

### 3.1. Cheese-making characteristics

During cheese manufacture, the gels made from milk treated with HP at low temperature were strong enough to cut after 16 and 18 min of rennet addition at 676 and 483 MPa, respectively, whereas gels from either raw or pasteurized milk were ready to cut 20 min after enzyme

addition. The decrease in clotting time observed in this work was similar to results in reconstituted low heat nonfat dry milk (NDM) reported by Desobry-Banon, Richard, and Hardy (1994), who found that treatments at pressures higher than 230 MPa enhanced acid and rennet coagulation in reconstituted low heat NDM. However, the current results differ from results reported by López-Fandiño et al. (1996). While they reported that coagulation time and the time needed for curd to become firm enough to cut decreased as pressure increased up to 200 MPa, coagulation and curdling times increased as pressures increased to 400 MPa, a result not significantly different from that reported for raw milk. The differences in rennet clotting time among these works could be due to temperature and storage effects. In López-Fandiño et al. (1996) work, the temperature of the vessel was controlled at 25 °C, with holding times of 10–60 min, whereas in the present work the initial temperature of the milk was 10 °C and pressurization was for 5 min. Furthermore, López-Fandiño et al. (1996) refrigerated the milk after pressurization and used it within 20 h of pressure treatment, whereas in the present work, cheeses were made immediately after pressure treatment. The procedures of the present study are more realistic for a real cheese processing scenario.

In contrast to the shorter coagulation times found at low temperature, when the temperature of the milk was increased to 30 °C prior to HP at 483 MPa, a very weak gel was formed. The curd was cut 30 min after rennet addition, as gel strength did not increase beyond this time. Milk heated to 40 °C prior to HP at 483 MPa did not form a gel, probably due to the formation of a complex between denatured  $\beta$ -lg and caseins that prevented rennet action. The formation of such a complex could be due to disulfide linkages (López-Fandiño et al., 1996; Needs et al., 2000). López-Fandiño and Olano (1998) also reported pressurization at temperatures higher than 40 °C affected coagulation adversely and prevented milk from coagulating. We

demonstrated that when the initial temperature of the milk is increased to 30 °C and pressurized at this temperature, the rennet clotting time changed drastically, from 18 min at 10 °C to more than 30 min at 30 °C, and that treatment at 30 °C resulted in a visibly weaker gel. Furthermore, when initial milk temperature increased to 40 °C no gel suitable for cheese-making was formed.

### 3.2. Composition of cheddar cheese

Composition of Cheddar cheeses for the various treatments is shown in Table 1. All cheeses, except for HP treatment at 483 MPa at 30 °C, fell within the US Standards for Cheddar cheese. Cheeses from milk treated at 483 MPa HP and 30 °C had average moisture of 40.16%, which is greater than the US Standard for Cheddar Cheese ( $\leq 39\%$ ) (CFR, 2002). Moisture of cheeses from raw, pasteurized or 483 MPa HP at 10 °C were not significantly different, but moisture for cheese made from milk treated at 676 MPa treatment was significantly higher than raw, pasteurized or 483 MPa HP at 10 °C. The gel formed at higher HP not only entrapped more water, but resisted syneresis. Drake, Harrison, Asplund, Barbosa-Cánovas, and Swanson (1997) reported that cheese made from cyclic HP treated (586 MPa) milk had 39% moisture, which was significantly different from cheeses made from pasteurized milk (37% moisture) and raw milk (37.6%). Reduced syneresis of curds made from skim milk have been reported for pressure treatments at 600 MPa and 25 °C (López-Fandiño et al., 1996). The increased moisture retention was suggested to be due to the formation of a finer structural network and to the water-binding properties of denatured  $\beta$ -lg incorporated into the protein matrix (Needs et al., 2000).

All cheeses complied with the US Standard for Cheddar cheese since they had more than 50% of milk fat by weight of solids (CFR, 2002). Cheese from raw, pasteurized,

Table 1  
Composition of Cheddar cheese made from milk subjected to different treatments

	Treatment					EMS <sup>1</sup>
	Raw	Pasteurized	HP 676 MPa at 10 °C	HP 483 MPa at 10 °C	HP 483 MPa at 30 °C	
Moisture (g/100 g)	35.26 <sup>a</sup>	35.96 <sup>a</sup>	37.76 <sup>b</sup>	35.80 <sup>a</sup>	40.16 <sup>c</sup>	0.243
MFFC <sup>2</sup> (g/100 g)	54.97 <sup>a</sup>	56.06 <sup>ac</sup>	57.50 <sup>bcd</sup>	55.55 <sup>ad</sup>	58.83 <sup>b</sup>	0.513
Fat (g/100 g)	35.88 <sup>a</sup>	35.86 <sup>a</sup>	34.33 <sup>a</sup>	35.56 <sup>a</sup>	31.72 <sup>b</sup>	0.358
FDM <sup>3</sup> (g/100 g)	55.02 <sup>ac</sup>	55.99 <sup>a</sup>	55.16 <sup>ac</sup>	55.38 <sup>ac</sup>	53.01 <sup>bc</sup>	0.777
Protein (g/100 g)	24.05 <sup>a</sup>	23.91 <sup>a</sup>	23.91 <sup>a</sup>	24.17 <sup>a</sup>	23.70 <sup>a</sup>	0.138
PDC <sup>4</sup> (g/100 g)	37.14 <sup>a</sup>	37.34 <sup>a</sup>	38.42 <sup>b</sup>	37.64 <sup>a</sup>	39.61 <sup>b</sup>	0.300
Salt (g/100 g)	1.14	1.12	1.28	1.28	1.22	0.005
SM <sup>5</sup> (g/100 g)	3.24	3.12	3.40	3.59	3.06	0.048

<sup>abcd</sup>Mean values in a row with different letters indicate significant differences at  $\alpha = 0.05$ .

<sup>1</sup>EMS: error mean square.

<sup>2</sup>MFFC: moisture in fat free cheese.

<sup>3</sup>FDM: fat in dry matter.

<sup>4</sup>PDC: protein in dry cheese.

<sup>5</sup>SM: salt in moisture exhibited no significant differences.

676 MPa HP at 10 °C and 483 MPa HP at 10 °C milk did not have significantly different values for fat in dry matter (FDM), whereas 483 MPa HP at 30 °C had FDM significantly lower fat than pasteurized milk cheeses, as a result of higher moisture retention. On a wet basis, protein content of the cheeses was not significantly different among treatments, however, when only solids were considered, i.e. protein in dry cheese (PDC), the difference became significant. PDC increased with HP treatment. Cheese from HP treatment at 483 MPa and 30 °C resulted in the highest PDC, significantly higher than cheeses made from raw, pasteurized, and HP 483 MPa at 10 °C. HP treatment at 483 MPa at 10 °C resulted in an intermediate PDC between the raw, pasteurized and HP 483 MPa at 30 °C. Hence, pressure increase promotes incorporation of whey proteins into the curd, and the extent of whey protein incorporation is dependent on pressurization temperature. Salt and salt in moisture (SM) contents of the cheeses were not significantly different among treatments.

Fat recovery among treatments varied from 90.97% to 95.62% (Table 2), but the differences were not significant. Protein recovery was not significantly different between cheeses made from raw or pasteurized milk, but was increased by pressure treatment (Table 2). In HP treatment 483 MPa at 10 °C, 77.28% of the protein was recovered. At this same pressure (483 MPa) when the treatment temperature increased to 30 °C, recovered protein increased to 81.55%. When the pressure was increased to 676 MPa, recovered protein was 79.90% and was not significantly different from the treatment at 483 MPa at 30 °C, which can be attributed to the synergistic effect of both temperature and pressure. Gaucheron et al. (1997) proposed that under the temperature and pressure conditions they studied (250 MPa, 40 °C), noncovalent interactions between denatured whey proteins and modified casein micelles would not be favorable. López-Fandiño et al. (1996) reported that pressurization of milk for 30 min at 300 and 400 MPa caused a substantial decrease in protein loss (7.5% and 15%, respectively) in the whey fraction, causing an estimated increase in the mean curd weight from

14% to 20%, respectively. Our results also confirm a significant increase in the actual yields due to the increase in protein and moisture content for 483 MPa at 30 °C and 676 MPa at 10 °C treatments.

### 3.3. Yield and yield efficiency

Cheese yields obtained for the various treatments are shown in Table 2. Wet yields were obtained immediately after pressing. The highest wet yield, 11.54%, was obtained for the cheese made from HP treatment at 483 MPa and 30 °C, and was not significantly different from the wet yield obtained for HP 676 MPa at 10 °C (11.40%). Drake et al. (1997) reported a similar wet weight yield (11.30%) for cheese made from milk treated at 586 MPa at 5 °C; the moisture content of cheese was 39.0%. Our cheese (676 MPa) had 11.40% wet weight yield and a moisture content of 37.76%. After HP treatment at 483 MPa and 10 °C, a lower wet yield was obtained (10.83%), but this value was not significantly different from the wet yield obtained for pasteurized milk (10.56%). Yield obtained for raw milk was the lowest (10.36%), and even though this value was not significantly different from the wet yield obtained for pasteurized milk, it was significantly different from the yield obtained for all HP treatments. Higher cheese yields have been previously reported (López-Fandiño et al., 1996) for HP treated milk, however the yield increase was determined by centrifugation of the curd and estimated from increased moisture retention in the curd and not by cheese-making.

Adjusted yields for a cheese with 37% moisture and 1.5% salt were calculated for comparison purposes among treatments and are shown in Table 2. There were no significant differences between adjusted yields for cheese made from raw or pasteurized milk. Adjusted yield for 483 MPa HP treatments at either temperature were not significantly different. Treatment at 676 MPa resulted in the highest adjusted yield (11.29%) and was significantly different from all other treatments except for the HP treatment at 483 MPa and 10 °C. Even though wet yields

Table 2

Actual, adjusted and theoretical yields obtained for cheese made from milk that received different treatments

Yield	Treatment					
	Raw	Pasteurized	HP 676 MPa at 10 °C	HP 483 MPa at 10 °C	HP 483 MPa at 30 °C	EMS <sup>1</sup>
Actual	10.32 <sup>a</sup>	10.56 <sup>ab</sup>	11.40 <sup>c</sup>	10.83 <sup>b</sup>	11.54 <sup>c</sup>	0.019
Adjusted	10.67 <sup>a</sup>	10.80 <sup>ab</sup>	11.29 <sup>c</sup>	11.08 <sup>bc</sup>	11.00 <sup>b</sup>	0.010
Theoretical	10.54 <sup>a</sup>	10.67 <sup>ab</sup>	11.06 <sup>bc</sup>	10.65 <sup>a</sup>	11.31 <sup>c</sup>	0.021
Actual vs. theoretical	-1.71 <sup>a</sup>	-1.03 <sup>a</sup>	3.07 <sup>b</sup>	1.69 <sup>ab</sup>	1.99 <sup>ab</sup>	2.191
<i>Recovery</i>						
Fat	91.22	93.18	95.62	94.76	90.97	
Protein	73.38 <sup>a</sup>	74.54 <sup>a</sup>	79.90 <sup>b</sup>	77.28 <sup>c</sup>	81.55 <sup>b</sup>	

<sup>abc</sup>Mean values in a row with different letters indicate significant differences at  $\alpha = 0.05$ .

<sup>1</sup>Error mean square.

for 676 MPa at 10 °C and 483 MPa at 30 °C were not significantly different, the adjusted yields were different. Hence, it is likely that the yield increase for 483 MPa at 30 °C HP treatment was due to a combination of increased protein recovery as well as increased moisture content, whereas at 676 MPa the yield increased mainly due to higher protein retention. López-Fandiño et al. (1996) reported that moisture retention and protein recovery at 400 MPa increased as holding time increased. PDC content for HP treatments at 483 MPa were significantly different depending on treatment temperature, with higher content at higher temperature. Our results show that treatment temperature plays a role, nearly as important as pressure, in affecting moisture retention and protein recovery in the curd, probably due to increased denaturation of whey proteins at higher temperatures. HP treatments involve adiabatic heating of the system that increases the temperature of the treated product by approximately 3 °C per 100 MPa, which can increase protein denaturation (López-Fandiño & Olano, 1998; Tauscher, 1995; Wick, Nienabert, Anggraeni, Shellhammer, & Courtney, 2004). Protein denaturation and disruption of hydrophobic interactions result in exposure of hydroxyl groups, which promote moisture retention (Drake et al., 1997; Johnston, Austin, & Murphy, 1992; López-Fandiño et al., 1996).

### 3.4. Amplitude sweep evaluation

The linear visco-elastic range was determined by evaluating the stress dependence of storage modulus ( $G'$ ) for cheeses from all treatments. All treatments showed similar behavior and a linear visco-elastic range was observed between 0 and 2 kPa, which agreed with the value reported by Ma, Drake, Barbosa-Cánovas, and Swanson (1997), for full and low-fat Cheddar cheese. A stress value of 1 kPa was selected for further rheological testing since lower stress indicated fluctuating values of  $G'$  and  $G''$  due to instrument sensitivity.

### 3.5. Frequency sweep evaluation

Frequency dependence of storage ( $G'$ ) and loss ( $G''$ ) moduli for cheeses made from all treatments are shown in Figs. 1 and 2, respectively. The  $G'$  is a measure of the energy stored and recovered per test cycle, whereas  $G''$  is a measure of the energy dissipated during a test cycle. In all cases, a larger elastic than viscous contribution to viscoelasticity ( $G' > G''$ ) was observed (i.e. more solid-like structure), and was consistent with prior reports (Messens, Van de Walle, Arevalo, Dewettinck, & Huyghebaert, 2002; Rosenberg, Wang, Chuang, & Shoemaker, 1995). The magnitude of storage and loss moduli of cheeses from all treatments was dependent on frequency and followed similar trends. Tukey's studentized range test for both,  $G'$  and  $G''$  were compared at shear angular frequencies of 173, 33 and 2.75 rad/s, respectively, and results are shown in Table 3. Significant differences were established at

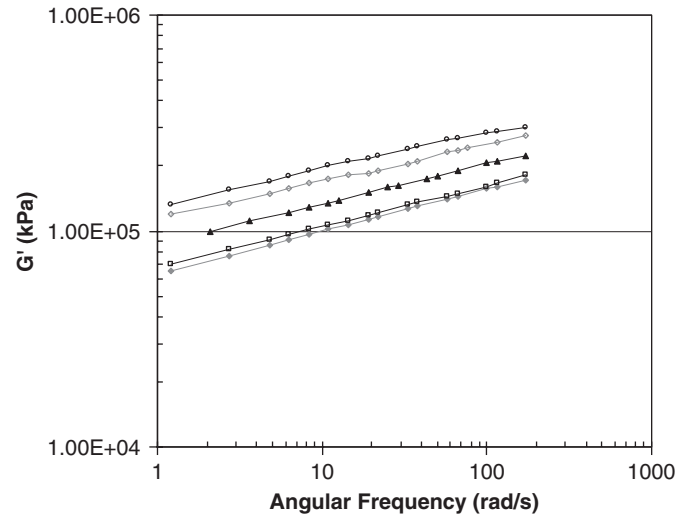


Fig. 1. Variation of storage modulus ( $G'$ ) with angular frequency for cheeses made from raw, HTST pasteurized and high pressure treated milk: (◇) raw milk; (◻) pasteurized milk; (●) HP 676 MPa at 10 °C; (○) HP 483 MPa at 10 °C; and (▲) HP 483 MPa at 30 °C.

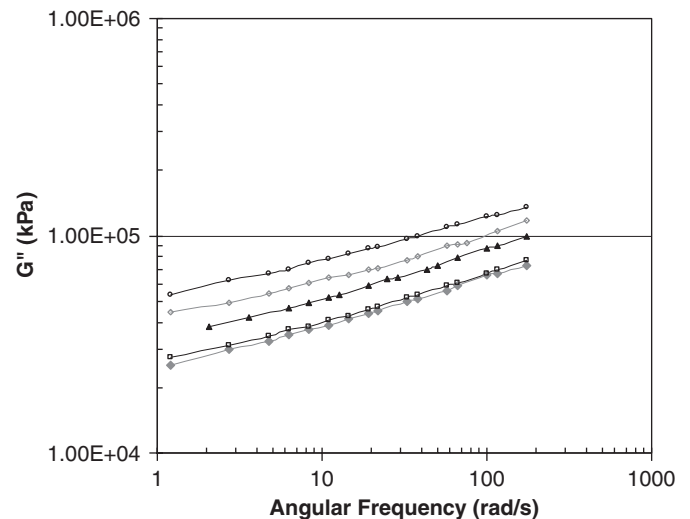


Fig. 2. Variation of loss modulus ( $G''$ ) with angular frequency for cheeses made from raw, HTST pasteurized and high pressure treated milk: (◇) raw milk; (◻) pasteurized milk; (●) HP 676 MPa at 10 °C; (○) HP 483 MPa at 10 °C; and (▲) HP 483 MPa at 30 °C.

$\alpha = 0.05$ . At a frequency of 173 rad/s the treatment 483 MPa HP at 10 °C produced a cheese with the highest  $G'$ , which was significantly different from all other treatments. The high  $G'$  can be attributed to the high protein and low moisture content compared to all other treatments (Table 1). When the frequency was decreased to 33 rad/s, milk treated 483 MPa HP at 10 °C was highest but no significant differences in  $G'$  were observed among cheeses from different treatments. When the frequency was further reduced to 2.75 rad/s, cheese from pasteurized milk had the lowest storage modulus (79.6 kPa), and cheese from 483 MPa HP at 10 °C milk showed the highest  $G'$  (154 kPa). Cheeses made from either raw milk or milk

Table 3  
Storage ( $G'$ ) and loss ( $G''$ ) moduli obtained from frequency sweeps for cheeses made from milk that received different treatments

Treatment	$G'$ (kPa)		$G''$ (kPa)		$\delta$ (deg)
	Mean*	SD	Mean*	SD	
<i>At 173 rad/s</i>					
Raw	229.0 <sup>a</sup>	11.7	97.5 <sup>a</sup>	5.0	23.0
Pasteurized	180.0 <sup>a</sup>	7.0	77.6 <sup>a</sup>	4.0	23.3
HP 676 MPa at 10 °C	232.0 <sup>a</sup>	12.3	98.1 <sup>a</sup>	1.5	22.9
HP 483 MPa at 10 °C	324.0 <sup>b</sup>	30.7	142 <sup>b</sup>	8.1	23.7
HP 483 MPa at 30 °C	195.0 <sup>a</sup>	19.1	86.7 <sup>a</sup>	13.2	23.9
<i>At 33 rad/s</i>					
Raw	140.0 <sup>a</sup>	19.5	56.1 <sup>ab</sup>	6.8	21.8
Pasteurized	130.0 <sup>a</sup>	4.7	52.0 <sup>a</sup>	7.1	21.8
HP 676 MPa at 10 °C	187.0 <sup>a</sup>	5.2	71.8 <sup>ab</sup>	14.7	21.0
HP 483 MPa at 10 °C	227.0 <sup>a</sup>	17.7	89.8 <sup>b</sup>	9.8	21.6
HP 483 MPa at 30 °C	206.0 <sup>a</sup>	20.0	76.1 <sup>ab</sup>	5.4	20.3
<i>At 2.75 rad/s</i>					
Raw	120.0 <sup>ab</sup>	28.1	44.9 <sup>ab</sup>	8.4	20.5
Pasteurized	79.6 <sup>a</sup>	3.6	31.6 <sup>a</sup>	5.2	21.7
HP 676 MPa at 10 °C	122.0 <sup>ab</sup>	15.9	45.2 <sup>ab</sup>	4.0	20.3
HP 483 MPa at 10 °C	154.0 <sup>b</sup>	23.8	58.3 <sup>b</sup>	9.0	20.7
HP 483 MPa at 30 °C	137.0 <sup>ab</sup>	6.0	48.8 <sup>ab</sup>	1.8	19.6

<sup>ab</sup>\*Mean values in a column with different letters in a common heading indicate significant differences at  $\alpha = 0.05$ .

$G'$ : storage modulus;  $G''$ : loss modulus;  $\delta$ : shift factor,  $\delta = \tan^{-1}(G''/G')$ ; SD: standard deviation.

treated with HP at 676 MPa at 10 °C or 483 MPa at 30 °C had  $G'$  values in-between 79.6 and 154 kPa and were not significantly different.

Because of the stronger protein gel, greater force was needed to deform the cheese made using 483 MPa HP at 10 °C treated milk. The  $G''$  of cheeses at high frequency (173 rad/s) followed a similar trend as observed for  $G'$ . The treatment 483 MPa at 10 °C had the greatest  $G''$ , which was significantly different from all other treatments. The high  $G''$  can be attributed to its high protein and low moisture content (Table 1). No significant differences were observed among  $G''$  values of 676 MPa HP at 10 °C, raw, pasteurized or 483 MPa HP at 30 °C. At lower frequency, i.e. 33 rad/s, pasteurized milk cheese showed the lowest  $G''$  (52.0 kPa) and 483 MPa HP at 10 °C exhibited the highest value of  $G''$  (89.8 kPa). Other treatments, i.e. HP 676 MPa at 10 °C, HP 483 MPa at 30 °C and raw milk cheeses fell within this range and were not significantly different from the two extreme values. At 2.75 rad/s the observed trend was similar to 33 rad/s, with pasteurized milk having the lowest  $G''$  value, HP 483 MPa at 10 °C the highest value, and the remaining treatments in-between, with no significant differences.

The  $G'$  and  $G''$  values are related to the type of network interactions, stable ( $G'$ ) vs. nonstable ( $G''$ ), during the course of the rheological measurement and, as a function of frequency, they are useful for comparing the effects of ingredients or processing treatments on visco-elasticity (Steffe, 1996). In the case of Gouda cheese, the relationship

between  $G'$  and  $G''$  has been suggested to depend on water content and proteolysis (Messens et al., 2002). In the present study, there is not an evident relationship between moisture content and  $G'$  and  $G''$  measured values. However, pressure treatment of milk (either at 483 or 676 MPa) increased milk protein recovery of the cheese, as shown in Table 2. Protein recovery in cheeses made with HP treated milk was higher than and significantly different from the protein recovery of raw or pasteurized cheeses. Even though at 676 MPa 10 °C and 483 MPa 30 °C the recovery was higher than and significantly different from the treatment at 483 MPa 10 °C, in the former case the increase in recovery was accompanied by higher moisture retention, whereas cheese moisture for the 483 MPa 10 °C treatment was not significantly different from either raw or pasteurized cheeses. Hence, it seems likely that the highest values of storage modulus observed for the 483 MPa 10 °C cheese at high frequency was related to higher protein recovery. Sensory evaluation studies are recommended for the future to evaluate if the impact has positive or negative implications.

### 3.6. Creep/recovery strain profiles

A characteristic of visco-elastic materials is that they undergo creep, i.e. they continue to deform under constant stress or loads (Purkayastha, Peleg, Johnson, & Normand, 1985). Creep and recovery responses for cheeses of all treatments are shown in Fig. 3 and the parameters obtained for each treatment are shown in Table 4. The instantaneous compliance ( $J_0$ ) may be related to the undisturbed protein network structure and is related to the rigidity of a material

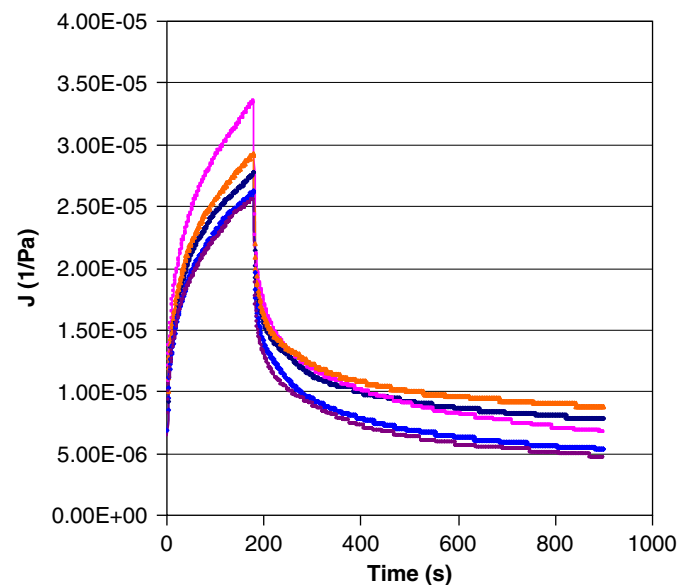


Fig. 3. Typical creep/compliance curves observed for Cheddar cheese made from raw, pasteurized, HP 676 MPa at 10 °C, HP 483 MPa at 10 °C and HP 483 MPa at 30 °C milk: (—) raw milk; (—) pasteurized milk; (—) HP 676 MPa at 10 °C; (—) HP 483 MPa at 10 °C; and (—) HP 483 MPa at 30 °C.

Table 4  
Creep/compliance parameters for Cheddar cheeses at 20 °C

Parameter	Raw	Pasteurized	HP 676 MPa at 10 °C	HP 483 MPa at 10 °C	HP 483 MPa at 30 °C
$J_{\max}$ (1/Pa) ( $\times 10^5$ )	2.89 <sup>ac</sup>	3.39 <sup>b</sup>	3.08 <sup>a</sup>	2.74 <sup>cd</sup>	2.55 <sup>d</sup>
$J_o$ (1/Pa) ( $\times 10^6$ )	9.01 <sup>ab</sup>	10.30 <sup>a</sup>	9.93 <sup>a</sup>	7.56 <sup>b</sup>	7.62 <sup>b</sup>
$J_m$ (1/Pa) ( $\times 10^5$ )	1.11 <sup>abc</sup>	1.21 <sup>a</sup>	1.14 <sup>ab</sup>	1.05 <sup>bc</sup>	0.99 <sup>c</sup>
$\lambda$ (s)	18.10	17.70	18.50	15.80	17.60
$\varepsilon_o$ (Pa s) ( $\times 10^{-7}$ )	1.97 <sup>a</sup>	1.49 <sup>b</sup>	1.80 <sup>ab</sup>	1.84 <sup>ab</sup>	2.14 <sup>a</sup>
$\gamma_o$ (%)	0.90 <sup>ab</sup>	1.03 <sup>a</sup>	0.99 <sup>a</sup>	0.77 <sup>b</sup>	0.75 <sup>b</sup>
$J_{eo}$ (1/Pa) ( $\times 10^5$ )	2.06 <sup>a</sup>	2.75 <sup>b</sup>	2.26 <sup>a</sup>	2.12 <sup>a</sup>	2.09 <sup>a</sup>
Elastic share of compliance (%)	71.30 <sup>a</sup>	81.20 <sup>ab</sup>	73.40 <sup>ab</sup>	77.50 <sup>ab</sup>	82.00 <sup>b</sup>
Viscous share of compliance (%)	28.70 <sup>a</sup>	18.80 <sup>ab</sup>	26.60 <sup>ab</sup>	22.50 <sup>ab</sup>	18.00 <sup>b</sup>

<sup>abc</sup>Mean values with different letters in a row indicate significant differences at  $\alpha = 0.05$ .

$J_{\max}$ : maximum compliance;  $J_o$ : instantaneous compliance;  $J_m$ : viscoelastic compliance;  $\lambda$ : mean retardation time;  $\varepsilon_o$ : zero shear viscosity;  $\gamma_o$ : instantaneous deformation;  $J_{eo}$ : steady-state compliance.

(Halim & Shoemaker, 1990). Cheeses from milk treated at 483 MPa at 10 °C exhibited the lowest instantaneous compliance ( $J_o$ ) of all treatments, except for 483 MPa at 30 °C, indicating a more rigid structure than cheeses from other treatments, which is in agreement with results obtained from frequency tests. We believe the results are supported by the fact that samples exhibited the highest protein content compared to all other treatments. Ma et al. (1997) compared the creep behavior of cheeses made from pasteurized milk and from low-fat milk with fat substitutes and demonstrated that full fat cheese (36.6% moisture) was more rigid than low fat cheeses (46.7% moisture). The differences in moisture content (37–51%) compared to ours (35–40%) likely explain these differences. In this study, we observed that cheeses from HP treated milk at 483 MPa at 10 °C possessed a more rigid structure than cheeses made with pasteurized milk, which is attributed to high protein and low moisture content of the cheeses. Cheese made from 676 MPa HP at 10 °C, with relatively high protein and higher moisture than 483 MPa HP at 10 °C, exhibited high  $J_o$ , indicating a low rigidity.

Maximum compliance ( $J_{\max}$ ) was higher for pasteurized cheese than for any other treatment. Cheese made with pasteurized milk showed the highest value of visco-elastic compliance ( $J_m$ ), although this value was not significantly different from the visco-elastic compliance observed for raw or 676 MPa HP at 10 °C cheeses. An increase in the visco-elastic compliance parameter ( $J_m$ ) is associated with a less solid-like and more visco-elastic behavior of cheese (Ma, Drake, Barbosa-Cánovas, & Swanson, 1996). The lowest  $J_m$  was shown by cheeses from 483 MPa HP at both temperatures, indicating a more solid-like behavior. As with the higher  $G'$  and  $G''$  values noted, the low  $J_m$  is supported by the high protein content of 483 MPa HP at 10 °C.

Residual strain is defined as the strain remaining after the recovery period, i.e. 720 s. Residual strains for cheeses are shown in Table 5. The highest residual strain values were shown by the raw and pasteurized cheeses and were not significantly different from any treatment except for the treatment HP 483 MPa at 30 °C. This can be attributed to

Table 5  
Tukey's studentized range test for creep and residual strains of Cheddar cheese

Treatment	Creep strain	Residual strain
	Mean	Mean
Raw	0.029 <sup>ac</sup>	0.0081 <sup>a</sup>
Pasteurized	0.034 <sup>b</sup>	0.0080 <sup>ab</sup>
HP 676 MPa at 10 °C	0.031 <sup>a</sup>	0.0063 <sup>a</sup>
HP 483 MPa at 10 °C	0.027 <sup>cd</sup>	0.0061 <sup>ab</sup>
HP 483 MPa at 30 °C	0.025 <sup>d</sup>	0.0045 <sup>b</sup>

<sup>abcd</sup>Mean values with different letters within a row indicate significant differences at  $\alpha = 0.05$ .

high PDC content of 483 MPa HP at 30 °C and increased cohesive force among proteins. The lowest residual strain exhibited by cheese HP 483 MPa at 30 °C, was not significantly different from HP 483 MPa at 10 °C or from pasteurized cheese. Further studies should be conducted to understand the effect of temperature on the composition and cheese yield and the impact of sensory properties, and acceptability.

#### 4. Conclusions

Milk treatment prior to cheese-making directly affects the final composition and yield of cheeses. Results from this research show that HP treatment at 10 °C significantly increases cheese yield and protein retention, as well as some of the rheological parameters compared to cheeses made from raw or pasteurized milk. HP treatment of milk at 676 MPa at 10 °C increased adjusted yield compared to pasteurization treatment, attributed to a combined action of protein and moisture retention. Dynamic oscillatory and creep/recovery tests confirmed association of high protein and moisture content and were useful in comparing the effects on cheese structure due to HP treatment of milk. The HP treatment 483 MPa at 40 °C did not result in gelation suitable to cheese-making. Our results suggest that pressurization temperature increases protein content and

moisture retention, most likely leading to cheese yield increase, at a temperature close to 10 °C.

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