Texture, proteolysis and viable lactic acid bacteria in commercial Cheddar cheeses treated with high pressure

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High pressure processing was investigated for controlling Cheddar cheese ripening. One-month- or 4-month-old Cheddar cheeses were subjected to pressures ranging from 200 to 800 MPa for 5 min at 25 °C. The number of viable *Lactococcus lactis* (starter) and *Lactobacillus* (nonstarter) cells decreased as pressure increased. Subsequent storage of the control and pressure-treated cheeses at 10 °C caused viable cell counts to change in some cases. Free amino acid content was monitored as an indicator of proteolysis. Cheeses treated with pressures ≥400 MPa evolved free amino acids at significantly lower rates than the control. No acceleration in free amino acid development was observed at lower pressures. Pressure treatment did not accelerate the rate of textural breakdown compared with the non-pressure treated control. On the contrary, pressure treatment at 800 MPa reduced the time-dependent texture changes. Results indicate that high pressure may be useful in arresting Cheddar cheese ripening.

Keywords: High pressure processing, cheese ripening, Cheddar cheese, *Lactococcus*, *Lactobacillus*.

Cheese ripening is an important step in the production of many cheeses. The ripening process can be lengthy for certain varieties, such as Cheddar (4–18 months). Ripening is a complex process involving microbiological, biochemical and physical changes. Enzymic degradation of carbohydrates, proteins and lipids is involved, as are secondary changes to the primary degradation products. Enzymes critical to this process come from a variety of sources: (1) indigenous milk enzymes, (2) rennet, (3) starter culture bacteria, and (4) nonstarter adventitious microflora. Proteolysis is considered the most important biochemical event in the development of Cheddar cheese flavour and texture (Fox et al. 1996).

Cheese manufacturers must produce an affordable product of consistent quality. Efforts to achieve this goal have included methods to accelerate the ripening process without adversely affecting cheese quality. Though progress has been made in accelerated ripening studies, greatly accelerated and balanced maturation of cheese remains difficult to achieve.

Cheese ripening continues during storage, distribution and retail of the product. Residual ripening can result in the consumer purchasing cheese with a stronger or different flavour than the manufacturer intended. The level of residual ripening depends on cheese manufacturing parameters and storage temperature and duration. Permanently arresting the microbiological and biochemical changes that occur during ripening could allow manufacturers to halt the ripening process at a desired stage, and thus, provide a more consistent product regardless of storage and distribution time. Arresting ripening may be particularly necessary when ripening-accelerating agents such as exogenous enzymes or adjunct cultures are added during cheese manufacture. These agents decrease the time required for ripening, but they may also continue to modify the cheese flavour after the desired endpoint is reached. Decreasing the temperature of storage can slow residual ripening, but no method currently exists to arrest cheese ripening.

Substantial evidence exists that pressure can activate or inactivate enzymes. At 'low' to 'medium' pressures (100–500 MPa) many enzymes display activation (Anese et al. 1995; Asaka & Hayashi, 1991; Cano et al. 1997; Weemaes et al. 1998), while at higher pressures (500–800 MPa) many of these same enzymes are inactivated (Cheftel, 1992; Goodner et al. 1998).

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Two previous reports describe the effects of pressure on Cheddar cheese ripening. A US and European patent exists which describes a process by which Cheddar cheese ripening is shortened from 6 months to 3 days through the application of pressure in the range of 10–250 MPa for 3 d (Yokoyama et al. 1992). O'Reilly et al. (2000) exposed Cheddar cheese to 50 MPa for 3 d and found a higher rate of proteolysis in very young cheeses during pressure treatment. Researchers have explored proteolysis in other cheese varieties post-pressure treatment. Messens et al. (2000a, 2001) attribute increased proteolysis of surface mould-ripened (Messens et al. 2001) and smear-ripened (Messens et al. 2000a) cheeses held at 50 MPa for 8 h to the increased pH observed in the pressure-treated cheeses compared with the control. In another study, proteolysis in Gouda cheese was unaffected by 50–400 MPa for 100 min or 50 MPa for 3 d (Messens et al. 1999). However, increased hydrolysis of β-casein was observed in Gouda cheese treated at 300 MPa for 30 min (Messens et al. 1998). Proteolysis in a goats' milk cheese was accelerated following exposure to 400 MPa for 5 min, though bitterness was detected in the pressure-treated cheeses (Saldo et al. 2000, 2002).

In the present study, five-minute applications of high pressure were explored as a method to control Cheddar cheese ripening. Briefly, low, medium and high pressure exposures (200–500 and 800 MPa) early in the ripening process were evaluated for accelerating cheese ripening. Exposures of ripened cheese to medium and high pressures (500–800 MPa) were assessed for arresting ripening.

Materials and Methods

Cheese

Two Cheddar cheeses of different ages were acquired from a major US cheese manufacturer as 40-pound (18·2 kg) blocks. A 1-month- and a 4-month-old cheese were obtained. Both cheeses were manufactured with a multiple strain *Lactococcus lactis* starter. No enzymes other than rennet were used in the manufacturing. The cheeses were cut into 1-pound (454 g) blocks of equal dimensions (6 × 6 × 11 cm) and vacuum packaged.

Cheese pressure treatment and storage

Each vacuum-packaged cheese block was subjected to a 5-min pressure treatment in the range of 200–800 MPa at 25±4 °C in a Quintus® High Pressure Food Processor (Flow Pressure Systems, Kent, WA, USA). The pressure transmitting fluid was one part distilled water: one part Houghto-Safe 620-TY (Houghton International, Valley Forge, PA, USA) and had an adiabatic temperature increase of approximately 3 deg C per 100 MPa. Pressure fluid and cheese samples were pre-conditioned to defined temperatures before the actual pressurization process to compensate for the adiabatic heat increase during pressure

come-up. These pre-pressurization temperatures ranged from 19 to 1 °C for pressure treatments from 200 to 800 MPa, respectively. Setting the temperature of the water-jacketed pressure chamber to the desired process temperature ensured constant process temperatures. Pressure, product temperature, and water-jacket temperature were monitored and recorded in 3 s intervals using a 21X Micrologger (Campbell Scientific Inc., Logan, Utah, USA) connected to a computer running PC208W datalogger support software (Campbell Scientific Inc., Logan, Utah, USA).

The 1-month-old cheese was subjected to pressures of 200, 300, 400, 500 or 800 MPa. The 4-month-old cheese was treated with 500, 600, 700, or 800 MPa. Non-pressure-treated controls for each cheese were held at atmospheric pressure (0·1 MPa) at 25 °C for 5 min. Cheeses were placed at 10 °C immediately after pressure treatment and stored at 10 °C until sampling. Cheeses were sampled at time zero (the day after pressure treatment) and at 2 or 4 week intervals up to 24 weeks after pressure treatment. Duplicate cheese blocks were evaluated for each pressure at each time point.

Enumeration of viable bacteria

An internal sample of each cheese was aseptically extracted and used to determine viability of the starter culture (*Lc. lactis*) and nonstarter *Lactobacillus* species. The sample was homogenized in sterile sodium citrate (20 g/l) and decimal dilutions were plated on M17-lactose (*Lc. lactis*) and Rogosa SL (*Lactobacillus*) agar plates (Difco, Detroit, MI). M17-lactose plates were incubated at 30 °C, and Rogosa SL plates were incubated anaerobically at 37 °C.

Free amino acid concentration

Free amino acid (FAA) content of the cheeses was monitored as an indicator of proteolysis over time post-pressure treatment. Five grams of cheese were extruded through a garlic press, 100 ml 62·5 mM-trisodium citrate at 50 °C were added, and the samples were shaken (225 rpm) at 50 °C for 1 h. The free amino acid concentration in the citrate dispersions was determined with the cadmium-ninhydrin method (Folkertsma & Fox, 1992) with modifications. The assay volumes were reduced, maintaining the same proportions, and absorbances at 490 nm were determined in a microtitre plate reader. Leucine solutions (0–5 mM) prepared in 62·5 mM-trisodium citrate were used as standards. Results are expressed in millimoles of free amino acids per gram of cheese. Analysis was performed in duplicate on each cheese block.

Uniaxial compression studies

Sample preparation and compression testing were performed using a protocol similar to that of Watkinson et al. (1997). Cheese blocks were equilibrated at 4 °C before sample preparation to aid in sample uniformity during core

removal. Cylindrical cheese cores 20 mm in diameter were prepared using a metal punch (lubricated with paraffin oil) attached to a manual drill press. Cores were taken in the same dimensional direction to avoid interference from anisotropy in the cheese blocks. Cores were cut on both ends with a pair of parallel, lubricated razor blades mounted 20 mm apart. Samples were stored in airtight plastic bags and equilibrated to 20 °C for approximately 2 h before compression testing.

A Texture Analyzer TA-XT2 (Stable Micro Systems, Surrey, England) was employed. Each 20 × 20 mm sample was placed centrally on an acrylic base plate. An acrylic plate of 100 mm diameter was used as a probe and initially positioned 25 mm above the base plate. Both plates were lubricated with paraffin oil to prevent adhesion of the cheese sample to the plate surfaces during compression testing. A crosshead speed of 1 mm/s was used, and the samples were compressed to 25% of their original height.

Raw data consisting of force (N) and distance measurements (mm) were acquired at a rate of 200 Hz. Distance and force data were converted to Hencky strain (dimensionless) and stress (kPa) using the following transformations (Watkinson et al. 1997):

$$\dot{\varepsilon}_0 = v/h_0 \tag{1}$$

$$\varepsilon_c = \Delta h_t/h_0 \tag{2}$$

$$\varepsilon_h = \ln(1+\varepsilon_c) \tag{3}$$

$$\sigma = \left(\frac{F_t}{\pi r_0^2}\right) \cdot (1-\varepsilon_c) \tag{4}$$

where v=speed of compression (mm/s); h_0=initial sample height (mm); Δh_t=displacement of crosshead at time t (mm); F_t=force from lubricated compression at time t (N); r_0=initial sample radius (mm); $\dot{\varepsilon}_0$=initial strain rate (s^{-1}); ε_c=Cauchy strain (dimensionless); ε_h=Hencky strain (dimensionless) and σ=stress from lubricated compression test (kPa).

Stress *v.* strain plots were prepared, and the point of maximum stress, the strain at this point, and the area under the stress-strain curve to this point were identified and/or calculated.

The point of maximum stress, where the material displays macroscopic failure, is termed the yield stress (Prentice, 1992), rupture stress (Mohsenin, 1986), or the fracture stress (Watkinson et al. 1997). This point, herein referred to as the fracture stress (kPa), was identified by examining the stress-strain data in Microsoft Excel (Seattle, WA, USA). The Hencky strain at this point, referred to as the fracture strain, was also identified. Due to the large deformation used in this method, the Hencky strain was used in place of a Cauchy strain (Steffe, 1996). The fracture work (kJ/m^3) was calculated as the area under the stress-strain curve up to the fracture stress. A fifth-order polynomial was fitted to the stress-strain data, and the coefficient of the linear term was used as an estimate of the curve's initial slope and hence the Young's Modulus (kPa). Six cores were obtained from each replicate block of cheese, and measurements made on each of the six samples were pooled together to obtain a value for that replicate.

Statistical analysis

For the microbiological and FAA data, differences among mean values at each time point were assessed using a one-way analysis of variance (SigmaStat® 5.0, SPSS Inc., Chicago, IL, USA). *P*-values $\leqslant 0.05$ were considered significant. Similarly, analysis of variance was performed using the GLM procedure of the SAS software (SAS, Version 8.02, Cary, NC, USA) to establish the influence of pressure treatment, storage time, and their interaction on the rheological parameters. The significant interactions between the independent variables (pressure treatment and storage time) were also reported. In all cases, a Tukey's mean test was used to identify the magnitude of differences among significant factors ($\alpha=0.05$).

Results

Bacterial counts in pressure treated cheeses

Increasing pressures reduced the viability of *Lc. lactis* and *Lactobacillus* in cheeses immediately after treatment (Figs. 1 and 2, Table 1). Subsequent storage of the control and pressure-treated cheeses at 10 °C caused viable cell counts to change in some cases.

For the cheeses pressure-treated after 1-month of aging, the untreated control cheese contained 3.8×10^8 cfu *Lc. lactis*/g on day 0 (Fig. 1A). The 200 MPa treatment did not reduce the *Lc. lactis* population significantly compared with the control, while pressures in excess of 200 MPa resulted in significant reductions in initial *Lc. lactis* populations. During post-pressure incubation at 10 °C, the *Lc. lactis* population began to decrease in the control and 200 MPa-treated cheeses after 84 d. The *Lc. lactis* population of the 300, 500 and 800 MPa-treated cheeses did not change significantly during incubation. The *Lc. lactis* population in the 400 MPa-treated cheese increased by 1.9 logs during the 24-week period, ending at the same level as the control, 200 and 300 MPa cheeses.

Quantifying *Lactobacillus* species assessed the presence of this genus of nonstarter lactic acid bacteria in the cheeses treated at 1-month of age (Fig. 1B). The non-pressure-treated control initially had 6.3×10^5 cfu *Lactobacillus*/g. All pressure treatments resulted in significant reductions in viable *Lactobacillus*. After incubation at 10 °C, the populations in the 0.1, 200 and 300 MPa-treated cheeses increased to 10^8 cfu/g by 42 d, then slowly declined to approximately 10^6 cfu/g. The 400 MPa-treated cheese reached 5×10^3 cfu/g at 42 to 56 days, after which counts declined to undetectable levels. Viable *Lactobacillus* cells in the 500 and 800 MPa-treated cheeses were below the detection limit of 10 cfu/g throughout the sampling period.
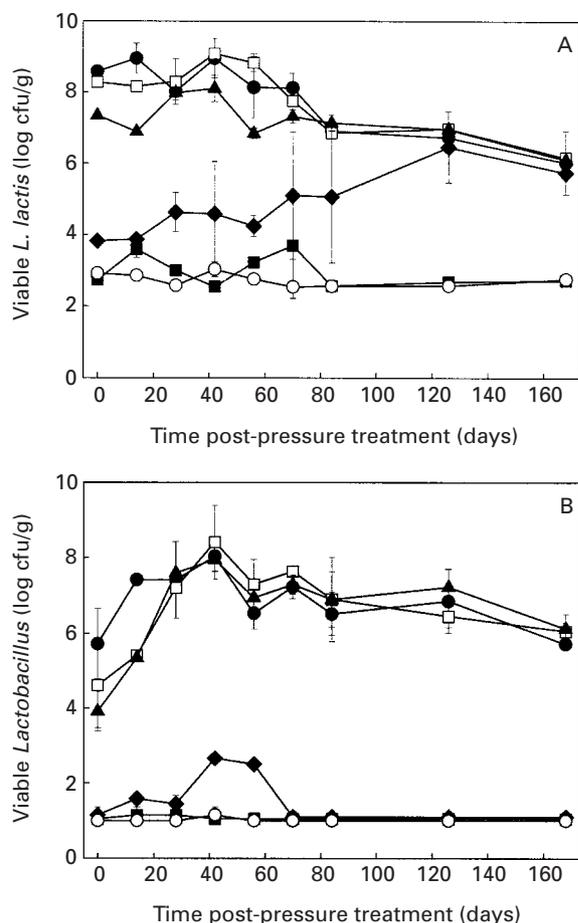
Fig. 1. Viable bacterial cell numbers post-pressure treatment in cheeses pressure-treated at 1 month of age and held at 10 °C for up to 24 weeks. A, *Lactococcus lactis* viable cells. B, *Lactobacillus* viable cells. ●, 0·1 MPa (atmospheric pressure control); □, 200 MPa; ▲, 300 MPa; ◆, 400 MPa; ■, 500 MPa; ○, 800 MPa. Detection limit was 1 log (cfu/g). Error bars represent +/− one standard deviation, *n*=2.

Table 1. Viable *Lactobacillus* in cheeses pressure treated at 4 months of age and held at 10 °C post-pressure treatment

Time†	log (cfu/g)‡				
(Days)	0 MPa	500 MPa	600 MPa	700 MPa	800 MPa
0	4·23	<1	<1	<1	<1
14	3·67	<1	<1	<1	<1
28	4·75	<1	<1	<1	<1
42	4·75	<1	<1	<1	<1
56	4·68	<1	<1	<1	<1
84	5·87	<1	<1	<1	<1
168	5·36	1·48	<1	1·40	<1

† Indicates time post-pressure treatment
‡ Detection limit was 1 log (cfu/g)
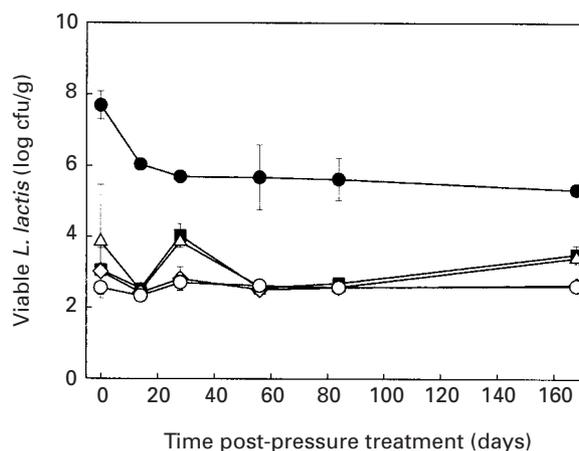Less than 10% variation was observed at each data point, *n*=2

Fig. 2. *Lactococcus lactis* viable cell numbers post-pressure treatment in cheeses pressure-treated at 4 months of age and held at 10 °C for up to 24 weeks. ●, 0·1 MPa (atmospheric pressure control); ■, 500 MPa; △, 600 MPa; ◇, 700 MPa; ○, 800 MPa. Detection limit was 1 log (cfu/g). Error bars represent +/− one standard deviation, *n*=2.

For cheeses pressurized at 4 months of age, the non-pressure-treated control contained $5·0 \times 10^7$ cfu *Lc. lactis*/g at the time of pressure treatment (Fig. 2). Pressure treatments resulted in significant reductions in starter culture levels. Slight increases in cell numbers occurred over time in moderate pressure treatments (500 and 600 MPa), while no changes occurred in the highest pressure treatments (700 and 800 MPa).

The non-pressure-treated 4-month-old cheese initially contained $1·7 \times 10^4$ cfu *Lactobacillus*/g (Table 1). This population increased by 1 log throughout the 24-week incubation at 10 °C. Pressure treatments of 500, 600, 700 and 800 MPa reduced the *Lactobacillus* population to undetectable levels (detection limit of 10 cfu/g) where it remained during incubation at 10 °C. Counts just above the detection limit were found in the 500 and 700 MPa samples after 24 weeks.

Free amino acid content

Free amino acid (FAA) content of the cheeses was monitored as a measurement of proteolysis in control and pressure-treated cheeses over time post-pressure treatment. Pressure treatment did not alter the initial FAA concentration significantly in any samples.

For the cheeses pressurized at 1 month of age (Fig. 3A), the FAA content of the 200 or 300 MPa samples did not differ significantly from the control throughout the sampling time. Cheeses treated with 400, 500 or 800 MPa evolved FAA at a significantly lower rate than the control, with 800 MPa retarding FAA evolution the most. No acceleration of FAA development, a marker for cheese ripening, was observed at any pressure level. Higher pressures delayed or arrested FAA development.

As expected, the FAA content of the 4-month-old control cheese (Fig. 3B) was higher at the time of pressure
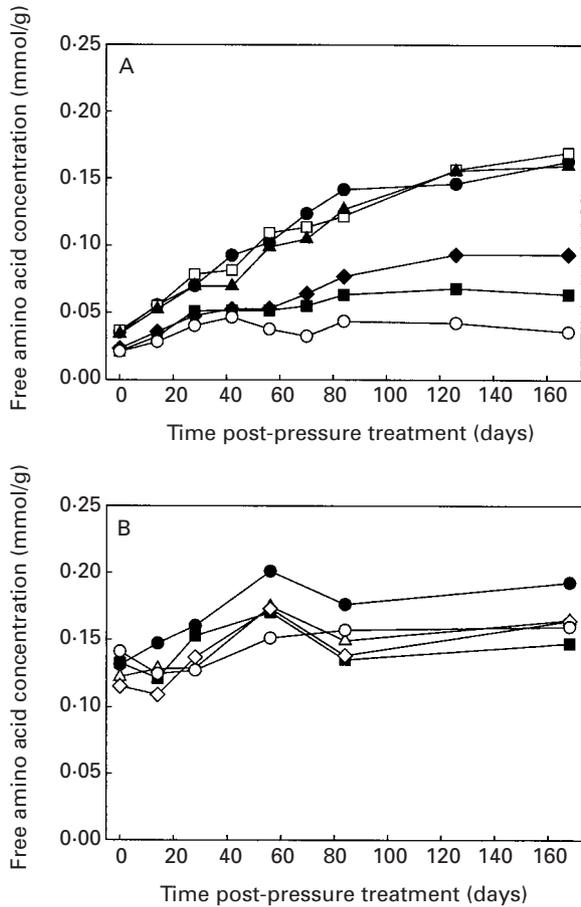
Fig. 3. Free amino acid concentrations in cheeses pressure treated at 1 month (A) and 4 months (B) of age and held at 10 °C for up to 24 weeks. Symbols are as described in Figs 1 and 2. Less than 10% variation was observed at each data point, $n=2$.

four-month-old cheeses. In both cases, the maximum and minimum CVs were 25·3% and 1·5%. Calculation of the Young's Modulus presented greater variability, with average CVs of 12·7% and 18·9% for the two respective trials.

Analysis of variance of the data in the first experiment, in which 1-month-old cheese was pressure treated, revealed significant effects due to pressure, storage time, and their interaction (Table 2). Across all pressure treatments, the fracture stress, strain and work decreased significantly over time, while the Young's modulus increased significantly with time. In general the samples became firmer but more crumbly with time, and moderate pressures did not appear to accelerate this process. The application of only moderate pressures up to 300 MPa had little effect on the cheeses' rheological properties compared with the control (Table 3). Similarly, the application of extremely high pressure treatments of 800 MPa resulted in cheese with similar fracture stress and Young's modulus across all storage times. The fracture work was significantly higher for the 800 MPa treatment but similar for all others (Fig. 4). The interaction between pressure treatment and time was apparent for the fracture stress, fracture work and Young's modulus (Fig. 5), where the 800 MPa treatment was similar to all other pressure treatments initially, but then became statistically different beyond 70 d storage.

The results for the cheese that was aged 4 months and then pressure treated indicated no significant effect on changes in rheological properties due to pressure, except for fracture work. Cheese rheology was affected primarily by storage time, and there were no pressure × storage time interactions as there were in the 1-month old cheese. All of the aged cheeses continued to firm with storage regardless of pressure treatment, and there was no apparent arresting effect achieved with the high pressure treatments. Pooled across all pressure magnitudes, there was little change in the fracture stress or Young's modulus until the last time point (Table 4). There was some mild variation in the fracture strain with a decreasing trend over time, until the last time point where there was another significant reduction in strain (Fig. 6). These decreases in strain were indicative of the cheese samples becoming more crumbly or fracturable with time. Fracture work showed no significant trend over time, until the last time point where the 800 MPa treated cheese showed a significant increase to $24·8 \pm 3·1$ kJ/m^3 (data not shown).

treatment than that of the 1-month-old cheese (0·13 and 0·044 mmol/g, respectively). The FAA content of the 4-month-old control cheese continued to increase during storage at 10 °C, however all pressure treatments (500–800 MPa) resulted in significantly lower FAA contents than the control beyond 56 d of storage ($P<0·05$), indicating that pressure treatment delayed or arrested free amino acid development in 4-month-old cheese.

Texture of pressure-treated cheeses

Due to the inherent inhomogeneity of biological materials, such as cheese, rheological measurements were performed on six samples from each replicate block of cheese and the results were pooled for use in statistical analysis. The variation in results within these six repeated measurements was not large overall. Expressed as the coefficient of variation (CV), the within replicate variability across all time points and measurements (except for the Young's Modulus) averaged 6·9% and 8·2% for the one-month-old and

Discussion

The present study demonstrates the effect of high pressure on viable lactic acid bacteria, FAA content, and texture of 1-month- and 4-month-old Cheddar cheeses. This is the first report of lactic acid bacteria viable counts in pressure-treated Cheddar cheese. The number of viable *Lc. lactis* and *Lactobacillus* cells decreased as pressure increased. Recovery and growth of cells during post-pressure incubation was evident in some cases. For example, *Lc. lactis*

Table 2. *Analysis of variance results for the rheological changes in the pressure treated cheeses as affected by pressure magnitude (P) and storage time (t), including degrees of freedom, F ratios, and means squared error (MSE)*

| | F ratios† | | | | | | | |
| | Cheese treated at one-month of age | | | | Cheese treated at four-months of age | | | |
	Pressure magnitude (P)	Storage time (t)	P × t	MSE	Pressure magnitude (P)	Storage time (t)	P × t	MSE
Fracture stress (kPa)	6·16***	5·67***	2·72***	32·9	1·38	13·35***	1·37	197·9
Fracture strain	10·23***	60·74***	2·12**	0·004	1·88	17·45***	1·25	0·002
Fracture work (kJ/m^3)	6·52***	29·55***	1·84*	8·08	3·30*	6·32**	1·39	5·42
Young's modulus (kPa)	5·49***	6·96***	3·01***	1326	1·59	4·82**	0·68	19701
Degrees of freedom	5	7	35	48	4	4	16	25

†*, significant at $P<0·05$; **, significant at $P<0·01$; ***, significant at $P<0·001$

Table 3. *Pressure-dependent rheological indices averaged across all storage times for one month old pressure treated cheeses*

Pressure treatment (MPa)	Fracture stress (kPa)	Fracture strain	Fracture work (kJ/m^3)	Young's modulus (kPa)
0·1	43·7±10·0^{A,B}	0·69±0·10^A	19·8±5·1^A	204±63^{A,B}
200	42·5±9·0^{A,B}	0·72±0·10^A	20·0±5·5^A	189±39^{A,B,C}
300	39·7±5·2^B	0·75±0·17^{A,B}	19·6±4·8^A	172±40^{A,B,C}
400	37·8±3·8^B	0·81±0·18^B	20·4±5·7^A	155±27^C
500	40·1±4·1^B	0·81±0·21^B	20·9±5·0^A	167±32^{B,C}
800	47·8±8·8^A	0·79±0·14^B	24·4±4·4^B	209±75^A

Means with the same superscripts are not different ($P>0·05$)
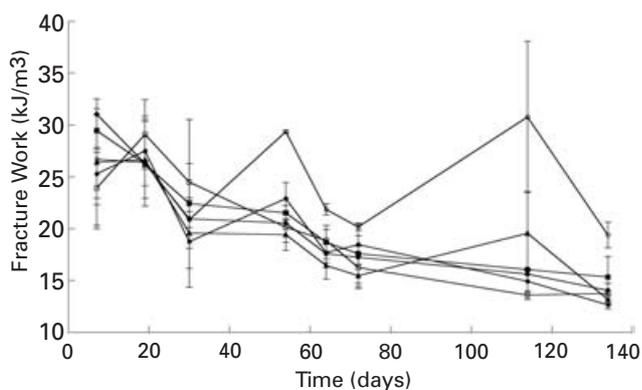
Fig. 4. Fracture work during compression of cheeses pressure treated at 1 month of age and held at 10 °C for up to 24 weeks. Symbols are as described in the Fig. 1 legend, $n=2$.
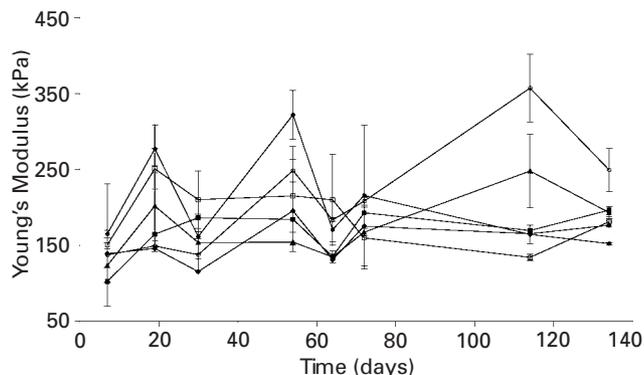
Fig. 5. Young's modulus of cheeses pressure treated at 1 month of age and held at 10 °C for up to 24 weeks. Symbols are as described in the Fig. 1 legend, $n=2$.

in 1-month-old cheese treated at 400 MPa was initially reduced by >4 logs, but grew to reach the same level as the control within 126 d (Fig. 1A). In other cases, cells did not increase in number after pressure treatment. Reductions in cfu/g were similar in 1-month and 4-month old cheeses at 500 and 800 MPa, the only pressures tested on both cheeses. Messens et al. (1999) reported a 5·5 log reduction in *Lc. lactis* in fresh Gouda cheese pressure treated at 400 MPa for 60 min, similar to the 4·7 log reduction observed in this study using 400 MPa for 5 min. This

suggests that increasing the duration of pressure exposure does not dramatically enhance lethality. *Lc. lactis* cells in buffer suspensions were completely inactivated at pressures ⩾400 MPa for 5 min (Malone et al. 2002). In the present study, viable *Lc. lactis* cells were recovered from cheeses treated with pressures up to 800 MPa. Therefore, the cheese matrix appears to protect the cells from the lethal effects of pressure.

Starter bacteria slowly die and autolyse during the ripening of Cheddar cheese. Autolysis releases intracellular peptidases that contribute to proteolysis. Malone et al.

Table 4. Time-dependent rheological indices averaged across all pressure treatments for four month old pressure treated cheeses

Storage time (days)	Fracture stress (kPa)	Fracture strain	Fracture work (kJ/m^3)	Young's modulus (kPa)
6	51·8±11·0^A	0·48±0·10^A	16·8±0·8^A	289±95^A
23	64·7±6·0^A	0·39±0·02^B	17·2±0·8^A	401±25^A
35	54·2±10·8^A	0·43±0·07^{A,B}	15·2±0·7^{A,B}	402±128^A
61	49·0±8·7^A	0·40±0·05^B	13·3±1·1^B	325±45^A
196	106·1±15·5^B	0·30±0·02^C	19·7±3·2^A	704±186^B

Means with the same superscripts are not different ($P>0·05$)
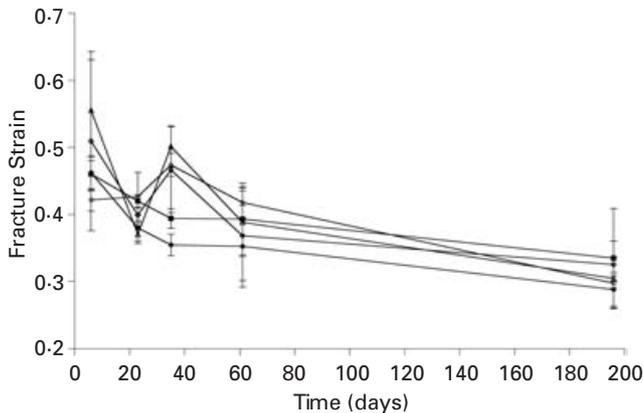

Fig. 6. Fracture strain during compression of cheeses pressure treated at 4 months of age and held at 10 °C for up to 24 weeks. Symbols are as described in the Fig. 2 legend, $n=2$.

(2002) demonstrated that high-pressure treatments (5 min, 100–800 MPa) promote autolysis of some *Lc. lactis* strains in cell suspensions. Thus, pressure has the potential to accelerate ripening by promoting release of intracellular peptidases. We did not observe acceleration of proteolysis in any of the pressure-treated cheeses in this study. In fact, cheeses with lower viable *Lc. lacti*s cell numbers displayed reduced proteolysis rates. This may be because autolysis is not promoted in the cheese matrix as it is in buffer suspensions or because the released peptidases are inactivated by pressure.

Nonstarter lactic acid bacteria gradually increase in number during cheese ripening. Non-starter bacteria can have a positive effect on cheese flavour; thus, the inactivation of *Lactobacillus* early in Cheddar cheese ripening may cause a dramatic change in cheese flavour development and proteolysis during ripening. The suppression of nonstarter *Lactobacillus* growth in cheeses treated at ≥400 MP may contribute to the reduced proteolysis rates observed.

We did not observe an increase in FAA concentration over time in any of the pressure treated cheeses compared with the control; thus, cheese ripening was not accelerated by the pressure conditions used in this study. However, higher pressures reduced the evolution of FAA regardless of cheese age, suggesting that short, high pressure applications may be useful in arresting cheese ripening at a

desired stage. At lower pressures (200 and 300 MPa), the rate of FAA release remained the same as the untreated control.

Researchers have attempted to accelerate proteolysis using various pressures and holding times. Accelerated proteolysis was observed in goats' milk cheese exposed to 400 MPa for 5 min (Saldo et al. 2000, 2002) and surface mould- and smear-ripened cheeses held at 50 MPa for 8 h (Messens et al. 2000a, 2001). Proteolysis in Gouda cheese was unaffected by 50–400 MPa for 100 min or 50 MPa for 3 d (Messens et al. 1999). Yokoyama et al. (1992) and O'Reilly et al. (2000) treated Cheddar cheeses at 50 MPa for 3 d and observed an acceleration of proteolysis. O'Reilly et al. (2000) observed accelerated proteolysis during pressure application for cheeses treated at 2, 7 and 14 d, but not for 21-, 28- and 92-day old cheeses. The rate of proteolysis during one month post-pressure treatment was the same as the control for all cheeses indicating that proteolysis was only accelerated during the pressure application and returned to the normal rate upon removal of pressure. We did not observe proteolysis acceleration when we exposed older Cheddar cheeses to higher pressures for shorter durations than the aforementioned study. Our data support the concept that proteolysis is only accelerated during the application of low pressures to very young cheeses.

The present study is the first to report slowing or arresting cheese proteolysis using pressure. Pressures ≥400 MPa decreased the rate of FAA evolution in the cheeses. Fewer viable lactic acid bacteria, enzyme inactivation, substrate alteration or a combination of these factors may cause decreased proteolysis in these cheeses. High pressure processing activates or inactivates proteases and peptidases depending on the specific enzyme and the pressure level (Miyakawa et al. 1994; Casal & Gómez, 1999; Malone et al. 2003). Malone et al. (2003) demonstrated that many cheese proteolytic enzymes are inactivated at pressures ≥400 MPa. Treatment at 300 MPa reduced the activity of some proteolytic enzymes, but 100 and 200 MPa did not reduce activity of any proteolytic enzymes studied. A few enzymes were activated or unaffected by high pressures. Inactivation of proteolytic enzymes may partially explain the reduced FAA concentrations observed in cheeses treated at ≥400 MPa. Pressure-induced conformational changes in the enzyme substrates may also contribute to reduced proteolysis.

Cheese is a non-linear viscoelastic material; consequently, the measured rheological properties are dependent upon the strain rate used during testing. While this study did not focus on viscoelastic property evaluation, the initial strain rate ($\dot{\varepsilon}_0 = 0.01\ \text{s}^{-1}$) for the compression testing was selected to be within the range used by other published studies on cheese fracture properties (Rohm & Lederer, 1992; Watkinson et al. 1997). Compression platen surfaces were lubricated to minimize frictional effects and reduce sample-to-sample variation due to testing conditions (Casiraghi et al. 1985). As the cheeses aged, variation within and among replicates increased because of breakdown in their physical structure that in turn hampered sample preparation. That is, samples taken from oldest cheeses tended to crumble when being cut, removed, and tested which lead to greater inter-sample variability with storage time.

Pressure treatment of commercially prepared 1- or 4-month-old cheese did not appear to accelerate the rate of textural breakdown due to the ripening process compared with the non-pressure treated control. The effects of extremely high pressure treatments (800 MPa) were variable. Pressure treatment at 800 MPa reduced the time-dependent changes in fracture work indicating that ripening may have been arrested. However, it had little effect on the fracture stress and Young's modulus relative to the non-pressure treated control. In all cases, the application of pressure did not adversely affect the rheological properties of the cheese.

The control cheeses' rheological development was similar to other studies on Cheddar cheese ripening (Watkinson et al. 1997). The results of this study confirm the trends in Cheddar cheese ripening using low pressures (<200 MPa) observed by other researchers. Published work on accelerating Cheddar cheese ripening, aimed at repeating the Yokoyama et al. (1992) patent using pressures of 50 MPa for extended periods of time throughout the ripening process, found only slight decreases in the processed cheeses' yield stress (O'Reilly et al. 2002). These differences were apparent at 14 and 28 days of ripening, but not at 2, 7, 21 and 92 days or ripening. Until now, no published data exist on the effects of extremely high pressures (800 MPa) on Cheddar cheese texture.

Mixed results have been found using other cheese systems. Messens et al. (2000b) found that the duration of Gouda cheese ripening had a greater influence on the cheeses' rheological properties than did the pressure process (225 and 400 MPa, 1 h, 14 °C). Large deformation stress relaxation and small strain oscillatory experiments confirmed that pressure-treated Gouda cheese had a weakened intermolecular structure compared with the non-pressure treated controls, but that the differences between the two groups diminished during ripening. Furthermore, texture profile analysis indicated no effect of pressure treatments on TPA hardness or fracture force following ripening. Goat cheese behaved differently. Saldo et al. (2000) examined goat cheese and found that pressure treatments up to 400 MPa for 5 min increased the elasticity

of cheese. The cooking properties of Mozzarella cheese were significantly improved following pressure treatment (O'Reilly et al. 2002). While the rheological properties of the intact Mozarella cheeses were not compared, pressure treatments of 400 MPa for 20 min at room temperature significantly reduced the melting time and increased the flowability and stretchability of the heated cheese.

High pressure processing offers a novel ripening control method that may be applicable to many cheese varieties. High pressure treatment alters the viable microbial flora, FAA evolution, and texture development in Cheddar cheese. The data presented suggest that short, high pressure exposures of $\geqslant 400$ MPa may be useful to arrest Cheddar cheese ripening.

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