3.2 HIGH HYDROSTATIC PRESSURE FOR ACCELERATING RIPENING OF GOAT'S MILK CHEESE: PROTEOLYSIS AND TEXTURE (2000) J. Saldo, E. Sendra, B. Guamis. *Journal of Food Science* 65 (4) 636-640

High Hydrostatic Pressure for Accelerating Ripening of Goat's Milk Cheese: Proteolysis and Texture

J. SALDO, E. SENDRA, AND B. GUAMIS

ABSTRACT: High hydrostatic pressurization is proposed for cheese ripening acceleration. Several treatments were used for accelerating ripening of goat's milk cheese: 50 MPa / 72 h, 400 MPa / 5 min and 400 MPa / 5 min followed by 50 MPa / 72 h all at 14 °C. Moisture content and pH were higher in 400 MPa treatments compared to the others. By measuring proteolysis indexes, 400 MPa treatments were found to accelerate ripening (14 d in contrast to 28 d conventionally) due to enhanced enzyme activity from inoculated starter culture. Sensory analysis indicated bitter notes in the accelerated ripened cheese. Pressurized cheeses were less crumbly and more elastic than control.

Key Words: goat's milk cheese, high pressure, ripening, proteolysis, texture

Introduction

RIPENING IS AN EXPENSIVE PART OF cheese production. CHEESE ripening involves product immobilization until the product reaches the optimum characteristics, large capital investment for maturing facilities, and weight losses in nonpackaged varieties, all of which increase ripening costs. Acceleration of ripening, especially in low-moisture, long-ripened varieties, is highly desirable, not only for economic reasons, but also to reduce risks associated with long aging periods. Most of the work in this field involves studies in ripening temperatures, addition of cheese slurries or exogenous enzymes, and use of modified starters and adjunct cultures. Advantages and limitations of these methods have been discussed by several researchers (Law 1984; Fox and others 1996). These methods aim to accelerate cheese ripening either by increasing levels of putative essential enzymes or by providing conditions to optimize "endogenous" enzymes activity.

A patent by Yokoyama and others (1992) describes a method for shortening ripening by application of high hydrostatic pressure (HHP) in association with a highly proteolytic starter and exogenous enzymes. Although the patent claims that results are comparable to the conventional 6 mo ripened cheese in only 3 days treatment, similar studies showed other conclusions. Reps and others (1998) found that pressures more than 600 to 800 MPa caused inactivation of proteases, and the treatment that produced the highest degree of proteolysis was at 50 MPa in Camembert, but in Gouda and Kurpiowski cheese there was no apparent influence of pressure. A panel of experts rated a 10 d old Camembert pressurized at 50 MPa for 4 h to be identical to a 14 d old untreated cheese. Messens and others (1998a) found that pressure treatment from 50 to 200 MPa does not influence the non-casein nitrogen fraction while the free amino acid content is slightly decreased at high pressures. Sendra and others (1999) found also slight increase in proteolysis when a 50 MPa treatment was applied to cheese. Jin and Harper (1996) described a method for reliable acceleration of ripening of cheese slurries by using HHP to maintain undesirable microorganisms under control, thereby enhancing flavor development.

High pressure accelerates ripening by increasing water retention, releasing bacterial enzymes, and increasing enzyme activity under pressure. According to the principle of Le Châtelier, the system tends to reach a new equilibrium where perturbation becomes partially compensated. A pressure increase causes an enhancement in processes associated with a decrease in reaction (or activation) volume. Pressure influences not only the equilibrium point of chemical reactions in solution, but also its reaction rate.

In this work, we present a new method for accelerating cheese ripening that involves an initial shock-treatment at 400 MPa for 5 min followed by a 50 MPa treatment for 72 h, to increase the activity of previously released enzymes.

The cheese object of this study is the most popular goat's milk cheese in Catalunya (Spain). It is produced by about 76 small-scale cheese makers with an annual production of 87,000 kg and with a billing of about 729,000 Euros/year (Pratginestos and Romero 1998). Garrotxa-type cheese is made by enzymatic coagulation using a homofermentative starter. Typical cheese size is about 1 kg, and about 2 mo ripening time is required. During ripening the cheese surface is covered by a dark gray mold, while its inside is white, with a compact cutting and creamy texture.

Results and Discussion

Composition

Composition of control cheese is similar to that described by Carballo and others (1994) for Valdeteja cheese, another Spanish hard goat's milk cheese.

pH in control and 50 decreased as a result of lactic acid bacteria activity (Fig. 1). pH value was higher in groups where treatment included pressurization at 400 MPa (see group designation in "Materials and Methods" section). Messens and others (1998) found a pH-shift of about 0.25 when a 400 MPa treatment is applied for 6 min in a Gouda cheese. This treatment reduced the microbiota slackening lactic acid production from lactose, and higher proteolysis (Fig. 1) contributed to pH increase. Saldo and others (2000) found a reduction in starter counts by about 3 log cycles as a result of 400 MPa treatment, causing a higher final pH. A reduction in acidifying activity in lactic acid cultures after HHP treatment has been found, even when there were no apparent loss of cell viability (Casal and Gómez 1999).

In spite of the differences in values of constituents between production samples, all the treatments showed the same tendencies. Water-loss rate was dramatically different between

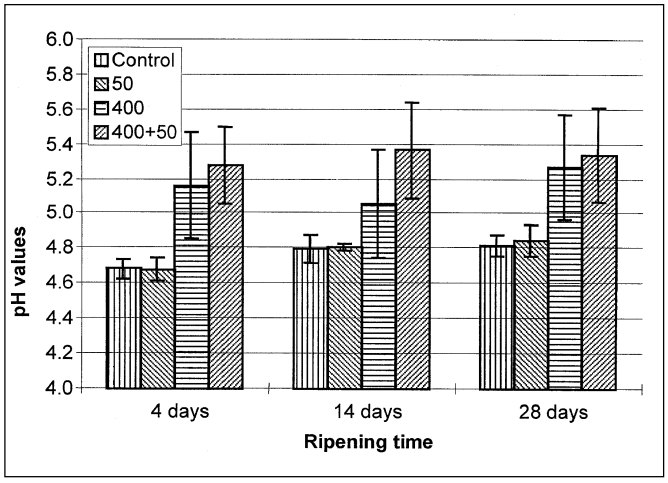

Fig. 1—Average pH values including standard deviation

treatments (Fig. 2a). Moisture retention was highest in treatment 400+50, followed by 400 and much lower in 50 and control. This could be related to higher proportion of small peptides due to the hydrolysis of casein by pressure (Fig. 2 C). This permitted water to be strongly bound. Dry matter content remained low, whereas dehydration was higher in nonpressurized groups.

Messens and others (1998) found similar differences in water retention between 300 MPa and untreated Gouda cheese, with differences increasing during aging.

Differences in non-casein nitrogen at d 4 are related to the increase in proteolytic activity of rennet during high-pressure treatment (Fig. 2 B). The treatment at 50 MPa for 72 h caused an increase in the amount of products of proteolysis at d 4, but during aging proteolysis was similar between 50 and control.

Proteolysis indexes were high in 400 MPa treated cheese, with the highest rate in 400+50. These results suggest that 50 MPa treatment would probably enhance proteolysis only while treatment is in progress, and 400 MPa causes an enzyme release that accelerates ripening. The same effect was observed in the evolution of non-casein nitrogen, nitrogen soluble in TCA, and free amino acids (Fig. 2 B, C, and D). Fox (1988) pointed out that the release of small protein fractions and free amino acids is mainly due to microbial enzymes, reinforcing the theory that the permeability of cellular membrane is increased by the high-pressure treatment (Earnshaw 1992; Cheftel 1992). Also most of starter peptidases have an optimal pH higher than that observed in control cheese (Visser 1993), and thus the increase in pH in 400 MPa treated cheese could have enhanced the enzymatic activity.

The decrease in the concentration of free amino acids after a 400 MPa treatment (Fig. 2 D) and in the nonprotein nitrogen (Fig. 2 C) could be related to an increase in the permeability of bacteria membrane that allows the small peptides and amino acids to enter into the cell and thus become nonsoluble and also nonquantifiable by ninhidrine method upon the soluble extract. Small peptides could also associate with bigger insoluble molecules through weak links or even disulfide bonds, but it is unlikely that single amino acids (except cistein) could associate in the same way. Yokohama and others (1992) reported a decrease in the amount of free amino acids, compared with untreated cheese, when pressure exceeded 300 MPa.

However, the overall level of proteolysis in terms of free amino acids, observed in Japanese cheddar cheese, even without application of HHP treatment, was substantially higher than that ob-

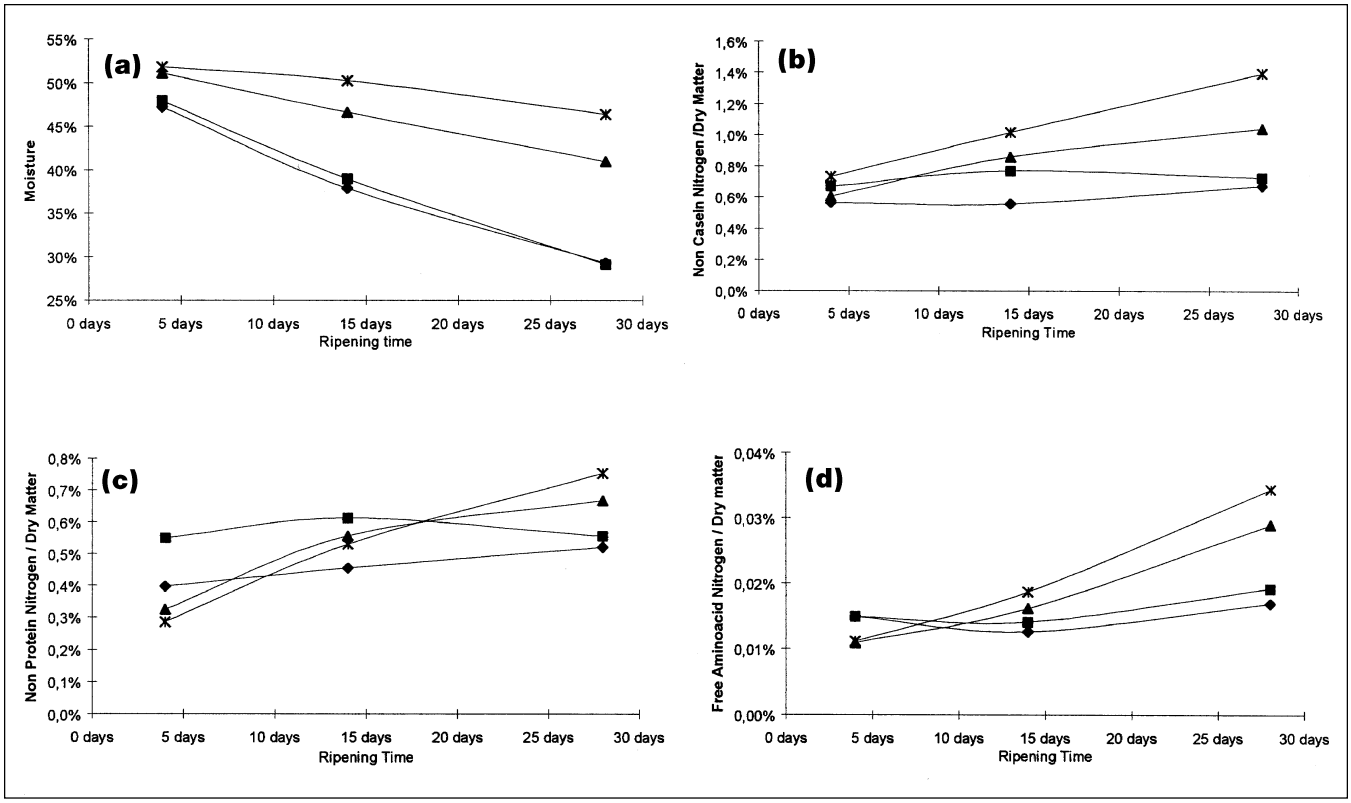

Fig. 2—Evolution of compositional values along the ripening time. Moisture and proteolysis products. ◆ = control cheese. ■ = 50 cheese. ▲ = 400 cheese. ✱ = 400 + 50 cheese.

Table 1—Nonlinear regression coefficients of the viscoelastic model 1 . Pooled correlation coefficient R^2 = 0.998.

	Ctrl	50	400	400 + 50
E_∞, kPa	156[a]	123[ac]	192[ab]	211[b]
E_1, kPa	215[a]	208[a]	271[ab]	290[b]
τ_1, s	9.19[a]	10.4a	7.16[b]	7.12[ab]
E_2, kPa	192[a]	181[a]	214[a]	226[a]
τ_2, s	105[a]	106[ab]	93.3[b]	94.2[b]

Mean values with common subscripts within each row do not differ significantly at P < 0.05.

Table 2—Viscosity coefficients

	Ctrl	50	400	400 + 50
η_1, Pa·s	1.9E + 06	1.9E + 06	1.8E + 06	2.0E + 06
η_2, Pa·s	1.9E + 07	1.7E + 07	1.9E + 07	2.1E + 07

The mean viscosity values are no different for a *P* < 0.05.

Table 3—Force-deformation parameters of 28-d-ripened "Garrotxa type" cheese, pressurized under different conditions

	Ctrl	50	400	400 + 50
E (kPa)	343[a]	278[b]	347[ac]	392[c]
ϵ_f (-)	0.237[a]	0.255[abc]	0.231[ac]	0.261[b]
σ_f (kPa)	64.8[a]	56.9[b]	63.2[ab]	80.2[c]
Wf (kJ/m^3)	6.53[a]	5.62[a]	6.07[a]	8.62[b]
$\sigma_{0.6}$ (kPa)	209[a]	167[b]	198[a]	210[a]
$W_{0.6}$ (kJ/m^3)	50.6[a]	41.9[b]	46.9[a]	47.9[ab]

Mean values with common subscripts within each row do not differ significantly at *P* < 0.05.

Table 4—Typical values of some parameters of a uniaxial compression test, for different cheeses. Adapted from Prentice (1987) and Almena and others (1998)

	E (kPa) -initial slope-	ϵ_f (-)	σ_f (kPa)
Edam	340 to 500	0.63	146
Gruyère	77	0.51	15
Cheddar	48 to 195	0.20 to 0.21	8 to 108
Arzúa-Ulloa	83	0.37	39
Gouda	270 to 405	0.37	69

served in commercial cheddar. The method of cheddar manufacture reported by Yokohama and others (1992) is substantially different from conventional Cheddar manufacture. In particular, starter addition to the milk was at least 10 fold higher than conventional inoculation rates.

Stress relaxation test

The decay in the force against the probe was fitted to the Maxwell's generalized model by nonlinear regression. We used a 5 element Maxwell model with 1 spring E_∞ in series with a parallel arrangement of 2 springs E_1 and E_2 each in series with dashpots η_1 and η_2, expressed by the following Eq.:

$$\frac{F(t)}{A \cdot \varepsilon} = E_\infty + E_1 \cdot e^{\left(-t/\tau_1\right)} + E_2 \cdot e^{\left(-t/\tau_2\right)} \quad (1)$$

where A is the compression area, ε is the true strain in the relaxation test, E_∞ is the equilibrium modulus of the elastic element, and E_1 and E_2 are the elasticity modulus, and τ_1 and τ_2 are the relaxation times for both Maxwellian elements in parallel.

The value of the elastic elements was greater and the relaxation times were longer for the 400 and 400+50 treatments than for control and 50 (Table 1).

Relaxation times (Table 2) were related to the viscosity coefficient (Pascals x second) in Maxwell's model (Kfoury and others 1989).

$$\eta_i = E_i \cdot \tau_i \quad (2)$$

The results can also be fitted to an exponential decay equation, where the force is expressed as an adimensional force, normalized respect to the initial force as suggested by Peleg (1979).

$$f(t)=\Delta F(t)/F(0)=c \cdot t^n \quad (3)$$

The pooled correlation coefficient for this model was R^2 = 0.91.

The correlation using the power model of Eq. 3 is poorer than the one using the Maxwell model of Eq. 1 but is good enough to be used. The model in Eq. 3 could be linealized and is easy to use. This empirical approach may sometimes be useful because there are only 2 parameters in the model, with c related with the force at equilibrium and n as a measure of the rate of decay.

The 50 treatments were no different from control, but 400 and 400+50 treatments showed a higher value of c and lower value of n than control (data not show). That means 400 and 400+50 cheeses were closer to fluid than solid behavior.

Uniaxial compression test

The strain was expressed as $\varepsilon_t = \Delta L/L_0$. The following parameters were calculated:

- deformability modulus (E, expressed in kPa), as the initial slope estimated by linear regression
- strain at fracture (ε_f)
- stress at fracture (σ_f) and at 0.6 strain ($\sigma_{0.6}$)
- work to fracture (W_f) and to 0.6 strain ($W_{0.6}$) were evaluated by the area under the stress-strain curve.

Stress is expressed as force by area. As the height of the sample is decreasing and the volume remains the same, the area must increase during the test.

Results shown in Table 3 are difficult to compare with those of other authors because the shape of the stress-strain curve depends on the compression rate and the sample height. Some typical values are shown in Table 4 for several cheeses. Bertola (1998) found in Pategrás Argentino cheese that σ_f directly depends on the integrity of casein matrix, and ε_f depends on water content and NPN. In our case, they depend on the HHP treatment despite the level of proteolysis. In 400 and 400+50, proteolysis is higher than control, so we can assume that the casein matrix is more degraded, but conversely those cheeses are more elastic than control, with the highest values for both σ_f and ε_f. After high-pressure treatment, cheese becomes more elastic and flows more easily (Messens and others 1998; Reps and others 1998; Saldo and others 1999). In a microstructural study performed by Capellas and others (1997), an even distribution of the protein network was observed in pressurized cheeses, and those changes in protein matrix could be responsible for changes in textural properties. Nevertheless, differences between pressurized and control cheese became less important with ripening time.

Textural changes induced by pressurization seem to be related to changes in Ca equilibrium and thus to the calcium-caseinate complex. Law and others (1998), working with goat's milk, found that calcium-casein associations were disrupted under high pressure because calcium migrates to the soluble phase. When pressure is released, the equilibrium is reestablished, but the association between caseins is not identical as it was previously. We propose that a similar mechanism occurs in cheese.

50 cheese was less elastic and easier to break than control. The less friable cheese was produced by means of the 400+50 treatment (Table 3).

Sensory

Sensory assessment by panelists evidenced bitter notes in HHP cheeses, especially in 400 (Fig. 3). Bitterness in cheese is a consequence of proteolysis, mainly associated to short hydrophobic peptides (Habibi-Najafi and Lee 1996). In our cheeses, bitter notes could be avoided by using starter cultures with reduced proteolytic activity and enhanced peptidolytic activity (Wilkinson and others 1994). Those cheeses were described as more acidic and less flavorful (except 400) than control ones. HPP cheeses were less crumbly, more elastic, and with higher mouth coating. Texture ratings coincided with instrumental results. From all attributes evaluated, panelists were more assertive in textural appreciations.

Panelists expressed no preference for any of the treatments used to prepare the cheese samples.

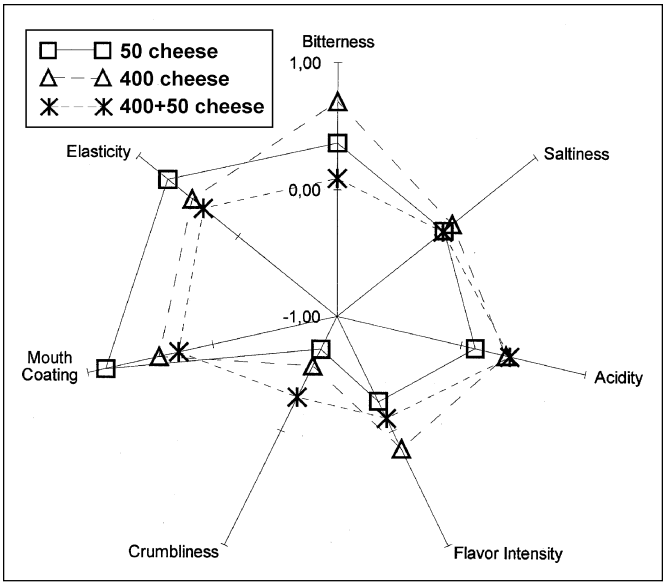

Fig. 3—Sensory profile. Differences from control.

Conclusions

COMBINATION OF SHOCK HIGH-PRESSURE TREATMENT 400 MPa/5 min to release microbial enzymes, followed by a long moderate-pressure treatment 50 MPa/72 h to enhance enzymatic activity can be a useful technology to accelerate cheese ripening. Water retention and proteolysis indexes are dramatically increased. Elasticity and mouth coating increased, and crumbliness decreased, but these textural changes did not affect panelists acceptability scores.

Further research is underway to find the mechanisms responsible for those changes and so define optimum conditions for cheese ripening acceleration.

Materials and Methods

Cheese manufacture

Garrotxa-type cheese was manufactured from goat's milk. Pasteurized (72 °C 15 sec) milk was inoculated (2% vol./vol.) with homofermentative starter (*Lactococcus lactis* ssp *cremoris*, *L. lactis* ssp *lactis*, *L. lactis* ssp *lactis biovar diacetylactis*). Calf rennet (0.07 ml/l, containing 780 mg chymosin/l) and calcium chloride (0.056 g/l) were used as coagulating agents. Coagulation occurred at 30 °C ± 1 °C within approximately 45 min, and then curd was gently cut into 8 to 10 mm cubes. The curd was held for 5 min before stirring and further warming up to 32 °C. When curds and whey reached the temperature, they were held for 15 min, then the whey was immediately drained from the vat. Drained curds were molded into cylindrical holders and pressed in a pneumatic press at 0.27 MPa for 30 min and then 0.55 MPa for 4 h. The molds were 5 cm deep by 9 cm dia and yielding pressed curds weighing about 250 g each. Cheeses were salted in brine (16% sodium chloride, 14 °C) for 45 min. Regular ripening conditions are 14 ± 1 °C and 86 ± 2% relative humidity.

Cheese packaging

All pieces were vacuum packaged individually in a low water and oxygen permeability barrier film (Cryovac BB4L, GRACE, Italy) to avoid contact between the cheese and the pressure transmission medium during the pressurization treatment. After 72 h packaging material was removed from all cheeses.

High-pressure treatment

The equipment used was a batch isostatic press from ACB Gec-Alshtom (Nantes, France) with a pressure vessel measuring 10 cm dia and 25 cm in length. Pressure vessel and water inside were kept at a treatment temperature of 14 °C by a constant flow of water through an external jacket. Temperature in the vessel was measured by a thermocouple.

Cheese groups

Cheeses were distributed in 4 groups: (1) control cheese, (2) cheese to be pressurized at 50 MPa for 72 h (50), (3) cheese pressurized at 400 MPa for 5 min (400), and (4) cheese pressurized at 400 MPa for 5 min plus 50 MPa for 72 h (400+50).

The treatment was carried out at 14 °C (the same temperature as the ripening chamber).

Chemical analysis

Cheeses were analyzed 72 h after processing and at d 14 and 28 of ripening. Each sample was analyzed for dry matter content IDF-FIL 1982 and pH (Marshall 1992). Proteolysis was evaluated by nitrogen fractions quantification: non-casein nitrogen (soluble at pH 4.6, acetate-acetic buffer) and nonprotein nitrogen (soluble in trichloroacetic acid 12%) (McSweeney and Fox 1997), and free amino acids (Folkertsma and Fox 1992).

Rheological measurements

Three units of 28 d old cheese were selected for each treatment from 2 independent productions. Cubic samples (10 mm side) were taken from the central zone. They were kept at room temperature (19 to 21 °C) for 2 h. Tests were performed with a TA XT2 Texture Analyzer (Stable Micro Systems, U.K.), and more than 10 cubes from each treatment and experience were tested.

Uniaxial compression was conduced at 60% compression using a plate of 50 mm dia and a crosshead speed of 5 mm/s.

Relaxation curves were obtained by compression until ε = 0.05, almost immediately (0.5 s), and maintained for 240 s.

Sensory evaluation

Sensory attributes were assessed by 23 panelists from our staff who rated differences against control (IFT 1981). Texture attributes such as crumbliness, mouth coating, and elasticity, and flavor attributes such as bitterness, saltiness, acidity, and flavor intensity were evaluated. Each evaluation was measured as the distance from the center where the control was the central point of a 140 mm scale. Panelists first tasted a reference cheese and then evaluated treated cheese and placed a mark in an unstructured scale. At the end of each trial, panelists performed a hedonic grading of cheese samples.

Statistical analysis

Means comparisons were done using a bilateral t-Student test for nonhomocedastic samples. The nonlinear regressions were performed with the STATISTICA software package (StatSoft 1995). The whole experience was run over 3 different cheese productions, with a control for each one. All the determinations were run in duplicate (except dry mater, in triplicate; and textural properties, 10 times).

References

Almena M, Noël Y, Cepeda A. 1998. Rheological characterization of Arzúa-Ulloa cheese by compression. Milchwissenschaft 53:316-319.

Bertola N, Califano A, Bevilacqua A, Zaritzky N. 1997. Efecto de la temperatura de maduración en la textura instrumental y proteolisis de queso Pategrás argentino madurado en película plástica. II Congreso Iberoamericano de Ingeniería de Alimentos. Tecnologías para el procesamiento y conservación de alimentos; 24-27 March 1997; Bahía Blanca, Argentina.

Capellas M, Mor-Mur M, Trujillo AJ, Sendra E, Guamis B. 1997. Microstructure of high pressure treated cheese studied by confocal scanning light microscopy. In: Heremans K., editor. High pressure research in the biosciences and biotechnology. Belgium: Leuven University Press. p 391-394.

Casal V, Gómez R. 1999. Effect of high pressure on the viability and enzymatic activity of mesophilic lactic bacteria isolated from caprine cheese. J Dairy Sci 82:1092-1098.

Carballo J, Fresno JM, Tuero JR, Prieto JG, Bernardo A, Martín-Sarmiento R. 1994. Characterization and biochemical changes during the ripening of a Spanish hard goat cheese. Food Chem 49:77-82.

Cheftel JC. 1992. Effects of high hydrostatic pressure on food constituents: an overview. In: Balny C, Hayashi R, Heremans K, Masson P, editors. High Pressure an Biotechnology. Vol. 224. London: Colloque INSERM/John Libbey Eurotext Ltd. p 195-209.

Earnshaw RG. 1992. High pressure as a cell sensitiser: new opportunities to increase the efficacy of preservation processes. In: Balny C, Hayashi R, HeremansK, Masson P, editors. High Pressure an Biotechnology. Vol. 224. London: Colloque INSERM/John Libbey Eurotext Ltd. p 261-267.

Folkertsma B, Fox PF. 1992. Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. J Dairy Res 59:217-224.

Fox, P.F. 1988. Acceleration of cheese ripening. Food Biotechnology, 2: 133-185

Fox PF, Wallace JM, Morgan S, Lynch CM, Niland EJ, Tobin J. 1996. Acceleration of cheese ripening. Antonie Van Leuveenhoek 70:271-297.

Habibi-Najafi MB, Lee BH. 1996. Bitterness in cheese: a review. Crit Rev Food Sci 36:387-441.

[IDF-FIL] International Dairy Federation–Federation Internationale de Laiterie. 1982. Fromages et fromages fondus. Détermination de l'extrait sec total (Métode de référence). International Dairy Federation Standard 4A. Belgium: Brussel.

[IFT] Institute of Food Technologists. 1981. Sensory evaluation guide for testing food and beverage products. J Food Sci 11:50-59.

Jin ZT, Harper WJ. 1996. Effects of high pressure treatment on changes of microflora and aroma profile in accelerated ripening of cheese slurry. J Dairy Sci 79 Suppl 1:114.

Kfoury M, Mpagana M, Hardy J. 1989. Effect of cheese ripening on rheological properties of Camembert and Saint-Paulin cheeses. Lait 69:137-149.

Law BA. 1984. The accelerated ripening of cheese. In: Davies FL, Law BA, editors. Advances in the Microbiology and Biochemistry of Cheese and Fermented Milk. London: Elsevier Applied Science Publishers. p 209-228.

Law JR, Leaver J, Felipe X, Ferragut V, Pla R, Guamis B. 1998. Comparison of the effects of high pressure and thermal treatments on the casein micelles in goat's milk. J Agric Food Chem 46:2523-2530.

Marshall, RT, editor. 1992. Standard methods for the examination of dairy products. 16th ed. Washington, D.C.: American Public Health Association. 546 p.

Messens W, Arevalo J, Dewettink K, Huyghebaert A. 1999. Proteolysis and viscoelastic properties of high pressure treated gouda cheese. In: Ludwing H, editor. Advances in High Pressure Bioscience and Biotechnology. Berlin: Springler-Verlag. p 445-448.

Messens W, Dewettinck K, Van Camp J, Huyghebaert A. 1998b. High pressure brining of Gouda cheese and its effect on the cheese serum. Lebensm.-Wiss. U.-Technol. 31:552-558.

McSweeney PLH, Fox PF. 1997. Chemical methods for the characterization of proteolysis in cheese during ripening. Lait 77:41-76.

Peleg M. 1979. Characterization of the stress relaxation curves of solid foods. J Food Sci 44:277-281.

Pratginestos F, Romero R. 1998. Producció actual del formatge de pell florida o Garrotxa. Pastors 14:10-12.

Prentice, J.H. 1987. Cheese Rheology. In: Fox PF, editor. Cheese. Chemistry, Physics and Microbiology. Essex, U.K.: Elsevier Applied Science Publishers Ltd. p 299-344

Reps A, Kolakowski P, Dajnowiec F. 1998. The effect of high pressure on microorganisms and enzymes of ripening cheeses. In: Isaacs NS, editor. High Pressure Food Science, Bioscience and Chemistry. Cambridge: The Royal Societyof Chemistry. p 265-270.

Saldo J, McSweeney PLH, Sendra E, Kelly AL, Guamis B. 2000. Changes in curd acidification caused by high pressure treatment. Irish Journal of Agricultural and Food Research: in press.

Saldo J, Sendra E, Guamis B. 1999. Changes in cheese texture and structure under long high pressure treatment. EFoST; 22-24 November 1999; Tampere, Finland.

Sendra E, Saldo J, Guamis B. 1999. Goat's milk cheese accelerated ripening. Compositional indexes. In: Ludwing H, editor. Advances in High Pressure Bioscience and Biotechnology. Berlin: Springler-Verlag. p 465-468.

StatSoft, Inc. 1995. STATISTICA for Windows [Computer program electronic manual]. Tulsa, Okla.

Visser S. 1993. Proteolytic enzymes and their relation to cheese ripening and flavor: an overview. J. Dairy Sci. 76: 329-350.

Wilkinson MG, Guinee TP, O'Callaghan DM, Fox PF. 1994. Autolysis and proteolysis in different strains of starter bacteria during Cheddar cheese ripening. J Dairy Res 61:249-262.

Yokoyama H, Sawamura N, Motobayashi N, inventors; Fuji Oil Company, Ltd., assignee; 05/02/1992. Method for accelerating cheese ripening. European-Patent-Application EP 0 469 851 A1.[DE, DK, NL].

MS 19990426 received 4/7/99; accepted 3/30/00.

This research has been supported by the European Commission, project FAIR-CT96-1113 (Project title: High Pressure Treatment of Liquid Foods and Derived Products). J. Saldo received a fellowship from the Catalan commission for Universities and Research.

Authors Saldo and Guamis are with the Planta de Tecnologia d'Aliments, UAB, CeRTA, Xit, Facultat de Veterinaria Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. Author Sendra is with the División de Tecnología de Alimentos, Universidad Miguel Hernández, Orihuela, Spain. Direct correspondence to Dr. B. Guamis (E-mail: buenaventura.guamis@uab.es).