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Effects of high-pressure treatment on free fatty acids release during ripening of ewes' milk cheese

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The free fatty acid (FFA) profile of high pressure treated ewes' milk cheeses were studied to assess the effect of pressure treatment on cheese lipolysis. Cheeses were treated at 200, 300, 400 or 500 MPa (2P to 5P) at two stages of ripening (after 1 and 15 days of manufacturing; P1 and P15) and FFA were assayed at 1, 15 and 60 d ripening. On the first day of ripening, 3P1-cheeses showed levels of FFA twice that of the control cheeses. However, no significant differences were found between 3P1 and control cheeses at 60 d ripening. On the contrary, 4P1 and 5P1-cheeses had the lowest total FFA levels. The point at which pressure treatment was applied influenced the FFA profile of cheeses; cheeses pressurized at pressures <400 MPa on the first day of ripening were more similar to untreated cheeses than their homologues treated at 15 d.

Keywords: High-pressure treatment, ewes' milk cheese, lipolysis.

Like proteolysis and glycolysis, lipolysis is an important biochemical event which takes place during the ripening of cheeses. The level of lipolysis varies considerably between the different cheese types; from low, in Dutch-type cheeses (Walstra et al. 1993), to extensive in the mould ripened, surface-bacterially ripened and Italian hard cheeses (Gripon, 1993; Reps, 1993). In other cheese varieties such as Manchego from ewes' milk, lipolysis is not very intense in quantitative terms, but it is qualitatively important since it plays a major role in the development of the characteristic aroma of this variety of cheese (Fernández-García et al. 1988).

Lipolysis releases free fatty acids (FFA) which contribute directly to cheese flavour, especially short- and intermediate-chain FFA. In addition FFA also act as substrates for further reactions producing highly flavoured catabolic end products (Collins et al. 2003a).

The lipolysis in cheese is catalyzed by a residual native milk lipoprotein lipase (LPL), rennet preparations containing pregastric esterase (PGE), lipase and esterase activities of starter lactic acid bacteria (LAB), non-starter LAB, adjunct cultures or secondary mould cultures, and exogenous lipolytic enzymes (Collins et al. 2003a; Wilkinson & Kilcawley, 2005). FFA can also be produced from the metabolism of carbohydrates and amino acids by bacteria (Urbach, 1993; Fox & Wallace, 1997).

The indigenous LPL, which is responsible to hydrolyses 1,3-positions in tri-, di- and mono-glycerides to give FFA, is nearly completely inactivated by HTST pasteurization (72 °C for 15 s; Andrews et al. 1987). However, it may contribute to lipolysis in pasteurized-milk cheeses as a time and temperature combination of 78 °C for 10 s is required for its complete inactivation (Driessen, 1989). The effect of rennet preparations on cheese lipolysis depends on the rennet type; commercial calf and bovine rennets are normally free from lipolytic activity (Georgala et al. 2005), but rennet pastes contain a lipase, pregastric esterase (Nelson et al. 1977). The effect of cheese microflora on lipolysis occurs via the esterase/lipase systems of lactic and propionic acid bacteria, non-starter LAB, surface microorganisms, yeasts and moulds (McSweeney & Sousa, 2000). LAB, especially *Lactococcus* and *Lactobacillus* spp. are weakly lipolytic (Fox & Stepaniak, 1993), however, they are responsible for the liberation of significant levels of FFA in many cheese varieties. To date, lipases/esterases of LAB appear to be exclusively intracellular (Chich et al. 1997). It has been suggest that they may require release into the cheese matrix through cell autolysis for maximum efficiency (Collins et al. 2003a). Lortal & Chapot-Chartier (2005) suggested a positive link between lysis of LAB and lipolysis.

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Ripening of cheeses is a long and costly process; consequently, new approaches to ripening acceleration have been attempted. Approaches which have been used include the addition of exogenous enzymes or cheese slurries to the curd, use of modified or novel starters or starter adjunct cultures, elevated ripening temperatures, and more recently the use of high pressure (HP; Law, 2001). Diverse research papers have reported that the application of HP on cheese can accelerate ripening, in particular proteolysis in cheese (Kolakowski et al. 1998; Messens et al. 2000; O'Reilly et al. 2003). However, available information about the effect of HP on lipolytic agents and cheese lipolysis is scarce. Inactivation of lipase (unspecified origin) was found above 700 MPa at 45 °C for 10 min in model tris buffer (pH=7), although after a treatment at 600 MPa and room temperature 60% of this activity was retained (Seyderhelm et al. 1996). Trujillo et al. (1999) found lipase activity in goat milk after a HP treatment of 500 MPa for 15 min. In addition, no inactivation of LPL was found up to 400 MPa for up to 100 min in milk (Pandey & Ramaswany, 2004), and some enhancement in its activity was found when pressures (300–400 MPa) were applied for a short time (Pandey & Ramaswany, 2004). Saldo et al. (2002) subjected goat milk cheeses to pressures of 400 MPa for 5 min and found lower amounts of FFA than control cheeses, suggesting that this HP-treatement decelerated lipolysis in these cheeses (Saldo et al. 2003). Lipolysis in Camembert cheese was only slightly affected by HP treatment of up to 500 MPa for 4 h, while lipolysis in Gouda cheese treated under the same conditions remained unaffected post-pressurization (Kolakowski et al. 1998).

There are various factors that influence the lipolysis in cheeses (release of intracellular lipase, the availability of fat and the activity of the enzymes) which could be affected by the pressure.

The objective of this study was to determine the FFA profiles of HP-treated (200 to 500 MPa) ewes' milk cheeses in order to evaluate the effect of HP on cheese lipolysis and the possible influence of ripening stage at which the treatment was applied.

Materials and Methods

Cheese manufacture

Cheeses (~0·5 kg) were manufactured from heat treated (75·5 °C, 1 min) ewes' milk. Milk was warmed at 38 °C and a starter culture containing *Lactococcus lactis* ssp *cremoris*, *Lc. lactis* ssp *lactis* and *Lc. lactis* ssp *lactis* biovar *diacetylactis* (Sacco SRL, CO, Italia) added to a final concentration of 1 l/100 l. A solution of $CaCl_2$ was added to cheese milk to a final concentration of 0·50 ml/l of a 350 g/l solution. Twenty minutes later, 0·2 ml calf rennet/l milk (Renifor-10, 520 mg chymosin/l, Lamirsa, Barcelona, Spain) was added. Temperature of coagulation was held at 38 °C and lasted about 30 min. The coagulum was cut into 8–10 mm cubes and the curd was drained, moulded, pressed (45 min at 1·2 kPa, 45 min at 1·8 kPa, 45 min at 2·45 kPa and 1 h at 3·1 kPa) and salted by immersion in brine (200 g NaCl/l solution) for 2 h. Cheeses were ripened in a room at 12 °C and 85% relative humidity for 60 d. The entire experiment was performed on two separate occasions.

High-pressure treatment

Cheeses were packed into vacuum pouches, vacuum-sealed and pressure-treated in a batch isostatic press (GEC Alsthom ACB, Nantes, France) at either 200, 300, 400 or 500 MPa (2P to 5P cheeses) for 10 min at 12 °C. One group of cheeses was treated on the first day (P1 cheeses) after manufacture and the others after 15 d (P15 cheeses) of ripening. Untreated cheeses were used as a control.

Compositional and microbiological analysis

Cheeses were analysed in triplicate for total solids (International Dairy Federation (IDF), 1982), fat (van Gulik method, ISO, 1975) and protein (IDF, 1993) and pH of cheeses were measured in duplicate with a pH meter (Crison Micro-pH 2001) on a cheese/distilled water (1 : 1) slurry. Microbiological analyses of total counts, lactococci and lactobacilli were performed as described by Juan et al. (2004). Analyses of cheeses were performed at 1, 15 and 60 d ripening.

Free fatty acid profile analysis

For the determinations of FFA at each stage of ripening, a sample of cheese (~200 g) was taken and stored at −80 °C until use, and thawed at 4 °C for 24 h before analysis.

FFA were extracted according to the modified method of De Jong & Badings (1990). Cheese (1 g) was placed in a screw-capped tube, and ground with 3 g anhydrous Na_2SO_4, 0·3 ml H_2SO_4 (2·5 m) and 30 µl internal standard solution (heptanoic acid 37·3 mg ml^{-1}). Diethyl ether-heptane (3 ml 1 : 1 v/v) was added and the mixture was shaken for 3 min using a vortex mixer. The supernatant was transferred to a screw-capped tube containing 1 g anhydrous Na_2SO_4. This operation was repeated three times. The lipid extract was applied to an aminopropyl column Spe-ed NH_2 500 mg ml^{-1}(Applied Separations, Allentown, PA, USA), which was conditioned with 10 ml heptane. Hexane/2-propanol (20 ml; 3 : 2 v/v) was applied to eliminate glycerides, and FFA were eluted with 5 ml diethyl ether containing 2% formic acid. A direct injection of this solution (1 µl) was used for gas chromatographic analysis. For each sample, two extractions of FFA were carried out.

Gas chromatography was carried out with a Hewlett Packard chromatograph equipped with an automatic on-column injector (HP 6890). A flame-ionization detector

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Table 1. pH values of control and HP-treated ewes' milk cheeses during ripening

Values are means for $n=4$

	HP treatment†								
Day	Control	2P1	3P1	4P1	5P1	2P15	3P15	4P15	5P15
1	$5{\cdot}30^{c}$	$5{\cdot}53^{a}$	$5{\cdot}59^{a}$	$5{\cdot}66^{a}$	$5{\cdot}6^{a}$	$5{\cdot}30^{c}$	$5{\cdot}30^{c}$	$5{\cdot}30^{c}$	$5{\cdot}30^{c}$
15	$5{\cdot}04^{c}$	$5{\cdot}08^{c}$	$5{\cdot}35^{b}$	$5{\cdot}55^{a}$	$5{\cdot}59^{a}$	$5{\cdot}05^{c}$	$5{\cdot}05^{c}$	$5{\cdot}06^{c}$	$5{\cdot}06^{c}$
60	$5{\cdot}09^{c}$	$5{\cdot}12^{bc}$	$5{\cdot}21^{b}$	$5{\cdot}01^{d}$	$5{\cdot}63^{a}$	$5{\cdot}13^{bc}$	$5{\cdot}09^{c}$	$5{\cdot}14^{bc}$	$5{\cdot}16^{bc}$

[a–d] Means in the same row followed by different letters are significantly different ($P \leqslant 0{\cdot}05$)
† Treatments: 2P–5P, cheeses treated at 200, 300, 400 and 500 MPa respectively; P1–P15, pressure applied on the 1st or 15th day of manufacturing

Table 2. Microbial counts (log cfu g^{-1} cheese) of control and HP-treated ewes' milk cheeses during ripening

Values are means for $n=4$

	HP treatment†								
	Control	2P1	3P1	4P1	5P1	2P15	3P15	4P15	5P15
Lactococci									
1	$8{\cdot}87^{a}$	$8{\cdot}34^{a}$	$8{\cdot}88^{a}$	$6{\cdot}77^{b}$	$4{\cdot}16^{c}$	$8{\cdot}87^{a}$	$8{\cdot}87^{a}$	$8{\cdot}87^{a}$	$8{\cdot}87^{a}$
15	$8{\cdot}25^{a}$	$8{\cdot}24^{a}$	$8{\cdot}71^{a}$	$7{\cdot}22^{b}$	$4{\cdot}28^{c}$	$8{\cdot}71^{a}$	$8{\cdot}48^{a}$	$6{\cdot}41^{b}$	$4{\cdot}53^{c}$
60	$8{\cdot}39^{a}$	$8{\cdot}97^{a}$	$8{\cdot}44^{a}$	$8{\cdot}19^{a}$	$4{\cdot}65^{c}$	$8{\cdot}30^{a}$	$7{\cdot}77^{ab}$	$7{\cdot}98^{ab}$	$4{\cdot}65^{b}$
Lactobacilli									
1	$3{\cdot}58^{a}$	$3{\cdot}71^{a}$	$2{\cdot}31^{b}$	$1{\cdot}87^{c}$	$1{\cdot}18^{c}$	$3{\cdot}58^{a}$	$3{\cdot}58^{a}$	$3{\cdot}58^{a}$	$3{\cdot}58^{a}$
15	$7{\cdot}46^{a}$	$7{\cdot}86^{a}$	$6{\cdot}70^{b}$	$6{\cdot}82^{b}$	$2{\cdot}16^{d}$	$7{\cdot}54^{a}$	$7{\cdot}19^{a}$	$4{\cdot}85^{c}$	$2{\cdot}17^{d}$
60	$7{\cdot}49^{ab}$	$8{\cdot}31^{a}$	$7{\cdot}90^{ab}$	$7{\cdot}80^{ab}$	$4{\cdot}16^{c}$	$7{\cdot}53^{ab}$	$7{\cdot}43^{ab}$	$7{\cdot}23^{ab}$	$3{\cdot}79^{c}$
Total counts									
1	$8{\cdot}73^{a}$	$8{\cdot}16^{a}$	$8{\cdot}11^{a}$	$5{\cdot}64^{b}$	$4{\cdot}32^{c}$	$8{\cdot}73^{a}$	$8{\cdot}73^{a}$	$8{\cdot}73^{a}$	$8{\cdot}73^{a}$
15	$8{\cdot}39^{a}$	$7{\cdot}98^{ab}$	$7{\cdot}20^{ab}$	$6{\cdot}91^{b}$	$4{\cdot}52^{c}$	$8{\cdot}31^{a}$	$7{\cdot}26^{ab}$	$6{\cdot}01^{b}$	$4{\cdot}95^{c}$
60	$8{\cdot}13^{a}$	$8{\cdot}49^{a}$	$8{\cdot}02^{a}$	$8{\cdot}02^{a}$	$4{\cdot}41^{c}$	$8{\cdot}20^{a}$	$7{\cdot}87^{b}$	$7{\cdot}78^{b}$	$4{\cdot}90^{c}$

[a–d] Means in the same row followed by different letters are significantly different ($P \leqslant 0{\cdot}05$)
† Treatments: 2P–5P, cheeses treated at 200, 300, 400 and 500 MPa respectively; P1–P15, pressure applied on the 1st or 15th day of manufacturing

(FID) was used either with a fused silica capillary column, 30 m × 0·25 mm i.d., coated with TRB-FFAP phase (df = 0·25). The carrier gas was high purity helium at flow rates of 0·9 ml min^{-1}. Temperature was raised from 50 to 240 °C at 5 deg C min^{-1} and then held at 240 °C for 20 min. The output signal from the detector was integrated using HP 6890 ChemStation software. Individual FFA were identified and quantified using standards supplied by Sigma (Sigma Chemical Company, St. Loius, MO, USA). Standard solutions with increasing concentrations of individual fatty acids and fixed concentrations of internal standards were used for the calculation of calibration curves.

Statistical analysis

Analysis of variance (ANOVA) was performed by SPSS Win version 12.0 (SPSS Inc., Michigan, USA), using 'HP' and 'the moment when treatment was applied' as the main factors at each ripening stage. Mean comparisons were carried out using the Student-Newman-Keuls test. Evaluations were based on a significance level of $P < 0{\cdot}05$.

Results

Compositional analysis

Cheeses presented 59·82 ± 0·71 % fat and 23·76 ± 2·58 % protein, both expressed on dry matter basis. Moisture content decreased during ripening in all cheeses (from about 45 % on the first day to 28 % at 60 d of ripening). The pH values of cheeses increased with pressure (Table 1), showing the highest values in 5P1-cheeses. The pH behaviour observed in 5P1-cheeses was due to the drastic reduction in LAB (Table 2) which are responsible for lactose fermentation and the production of lactic acid in the initial stages of ripening. P15-cheeses presented similar pH values to control cheeses, indicating that at 15 d ripening the acidification process was finished.

A significant decrease of total microbial counts was obtained at ⩾400 MPa (Table 2). During ripening,

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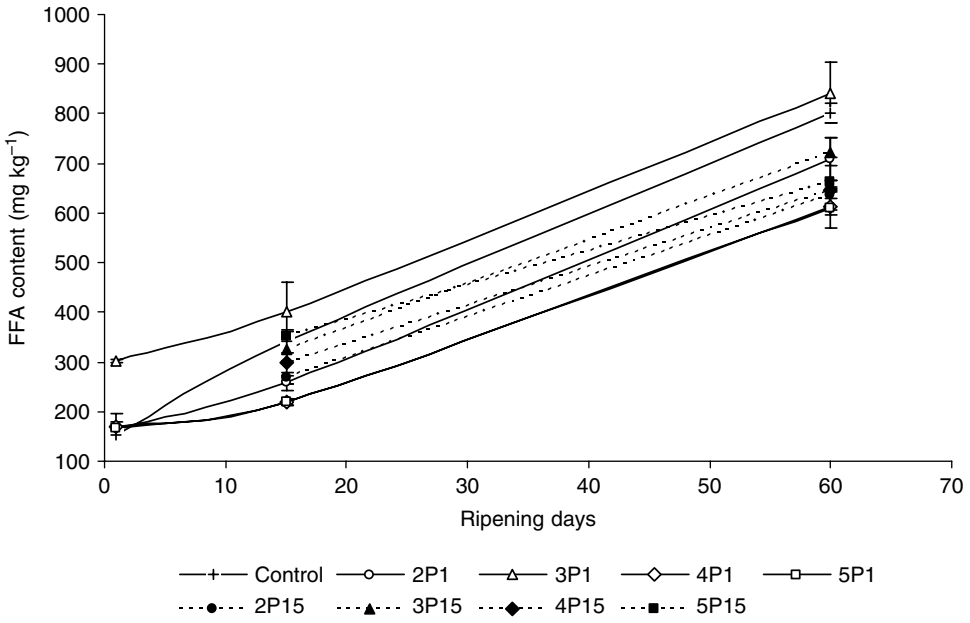

Fig. 1. Changes in the total free fatty acids content during ripening in control (+), 200 MPa (○), 300 MPa (△),400 MPa (◇) and 500 MPa (□) pressure treated ewes' milk cheeses. Open symbols with solid line represent P1-cheeses (pressure applied d 1); closed symbols with dotted line represent P15-cheeses (pressure applied d 15).

4P-cheeses recovered their microbial counts, reaching similar values to control cheeses at the end of ripening (8 log cfu/g). However, counts of 5P-cheeses did not recover during ripening and presented the lowest values (4 log cfu/g) at all times studied (Table 2).

FFA analysis

FFA content of cheeses increased from d 1 to d 60 (Fig. 1), indicating the significant effect of ripening time on cheese lipolysis. Total levels of FFA at 60 d ripening were similar to those reported for Teleme cheeses made from ewes' milk (Mallatou et al. 2003), but lower than those found by Poveda et al. (2000) in Manchego cheeses elaborated from raw milk.

The relative increase of the total FFA from 15 to 60 d ripening was according to the moment at which cheeses were HP treated. Cheeses pressurized on d 1 show higher FFA released than those pressurized on d 15 (Fig. 1). The concentrations of each of the 10 individual FFA, from $C_{4:0}$ to $C_{18:2}$ throughout the ripening are shown in Table 3. $C_{16:0}$ was the main FFA for all cheeses at all stages of ripening, coinciding with the literature (Kondyli & Katsiari, 2001; Mallatou et al. 2003; Fernández-García et al. 2006).

The ratios of short chain FFA (SCFA, $C_{4:0}$–$C_{8:0}$), medium-chain FFA (MCFA, $C_{10:0}$–$C_{14:0}$) and long chain FFA (LCFA, $C_{16:0}$–$C_{18:2}$) to total (T)FFA in cheeses varied with the ripening time. The SCFA/TFFA and LCFA/TFFA ratios increased and decreased, respectively during aging, while the MCFA/TFFA ratio slightly decreased with ripening. Similar results were found in Idiazábal (Chávarri et al. 1999), Manchego and Zamorano cheeses (Fernández-García et al. 2006).

Differences in the FFA profile were observed between cheeses due to the effect of HP treatment. At 1 d ripening (Table 3a), the highest levels of all individual FFA were detected in the 3P1-cheeses which also showed the highest levels of TFFA.

Higher values of $C_{10:0}$ and $C_{12:0}$ acids were found in HP-treated cheeses at pressures >200 MPa, compared with control and 2P1-cheeses, which in turn had similar values of all FFA.

At 15 d ripening (Table 3b), the highest amounts of TFFA were observed in 3P1-cheeses, which had the highest levels of $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$ and $C_{18:1}$ acids. HP-treated cheeses had lower levels of $C_{6:0}$ than control cheeses. However, among the HP-treated cheeses, those pressurized at 300 MPa had the highest amount of $C_{6:0}$. The same occurred with $C_{8:0}$, where control and 3P-cheeses presented the highest values. The lowest values of the main individual FFA were found in 4P1 and 5P1-cheeses.

The highest LCFA values were found in 3P1 and 5P15-cheeses essentially due to the great amounts of $C_{16:0}$ and $C_{18:0}$, respectively.

At the end of ripening (Table 3c), the highest values of TFFA were found in control and 3P1-cheeses. In contrast, 4P1 and 5P1-cheeses exhibited the lowest concentrations. Moreover, 5P1-cheeses showed the lowest amounts of the main individual FFA with the exception of $C_{4:0}$, which had the highest relative increase from 15 to 60 d ripening,

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probably due to its lower catabolism to other aromatic compounds. FFA act as precursor molecules for a series of catabolic reactions which lead to the productions of other flavour components (Collins et al. 2003a). In a previous study, we observed that HP treatment modified the volatile profile of cheeses, enhancing or limiting the metabolic pathways of some compounds (Juan et al. 2007a).

At the end of ripening, the highest levels of $C_{8:0}$, $C_{10:0}$, $C_{14:0}$ and $C_{18:1}$ FFA were found in 3P1-cheeses.

Discussion

Lipolysis in cheese is attributed to the action of lipases from milk, rennet, starter and non-starter bacteria. In this study the heat treatment applied to cheese milk (75·5 °C, 1 min) is able to inactivate indigenous milk lipase. Furthermore, commercial calf rennet free from lipolytic activity has been used. For that reason, the development of lipolysis in these cheeses was basically due to microbial lipases activity.

The different behaviour found in P1 cheeses could be attributed to different phenomena acting in HP processing of cheese. The main changes caused by HP application implicated in lipolysis would be inactivation of micro-organisms and lysis accompanied by enzyme release, and modification of protein conformation which have repercussion in enzyme activity, accessibility to substrates and modifications in the curd network (O'Reilly et al. 2001). The relative importance of these aspects is dependent on the magnitude of pressure application. In this study, lipolysis in early stages of ripening is enhanced by HP treatment of 300 MPa on the first day after cheese manufacture, indicating the relative importance of these factors and the way in which these different forces converge positively at this pressure to promote lipolysis.

The location of most microbial lipolytic enzymes appears to be intracellular (Collins et al. 2003b), and for this reason it is evident that they may require release into the cheese matrix through cell lysis for maximum efficiency. Khalid & Marth (1990) reported that certain strains of lactobacilli are able to liberate high activity of intracellular lipase upon autolysis, which may account for the great lipolysis observed during ripening of the cheese variety studied. HP treatment could favour the lysis of starter bacteria enhancing the release of intracellular lipases into the cheese matrix. Malone et al. (2002) showed that cell lysis of *Lc. lactis* subsp. *cremoris* MG1363 increased with increasing pressure up to 300 MPa, decreasing thereafter. Therefore, it seems to be that intermediate pressure levels promote lysis to a greater extent than the lower or higher pressures. In a previous study we have observed an increase of lysis with an increase of pressure, 3P1-cheeses showing the highest LDH activity at 15 d (Juan et al. 2007b). Moreover, although no differences in the lactococci counts were found between control and 3P1-cheeses at 1 d ripening, a decrease of 1 log unit of lactobacilli was found in 3P1-cheeses after the HP treatment. Consequently, the higher rates of lipolysis found in 3P1-cheeses at 1 d ripening could be attributed to the faster release of intracellular enzymes into the cheese induced by the pressure treatment.

Pressures $\geqslant$400 MPa applied on d 1 ripening decelerated the lipolysis in cheeses. The higher pressures reduced counts of starter and non-starter bacteria, with a drastic reduction by 500 MPa. The lower counts of micro-organisms might explain the lower amount of FFA found in these cheeses. Furthermore, although starter cells in cheeses treated at higher pressures ($\geqslant$400 MPa) could cause a more extensively lysis than those of the control cheeses, these higher pressures could inactivate enzymes (Simpson & Gilmour, 1997) limiting cheese lipolysis. Lower amounts of $C_{4:0}$, $C_{6:0}$ and $C_{8:0}$ acids were found in goat milk cheeses HP-treated at 400 MPa for 5 min compared with untreated cheeses (Saldo et al. 2003). These results were attributed to starter counts diminution and inactivation of lipolytic enzymes from the secondary microbiota, which reduced the amount of fatty acids released. Furthermore, the supramolecular structure of fat in cheese is greatly influenced by the treatment applied (López, 2005). HP-induced changes in the microstructure of cheeses (Kunugi, 1992) could alter the accessibility of lipolytic enzymes to fat. In this respect, O'Reilly et al. (2003) showed structural differences between Cheddar treated at 350–400 MPa and control cheeses. HP-treated cheeses (350 MPa for 70 h or 400 MPa for 42·5 h) presented the fat that was more emulsified and appeared to be encapsulated by the protein. The protein phase was more continuous after pressure treatment and thus more effective in trapping the fat. Similar observations were found by Juan et al. (2007c) in a textural and rheological study of these HP-treated cheeses from ewes' milk. HP application caused a more homogeneous and continuous cheese matrix which had repercussions in mechanical properties, and hence in microstructural characteristics. At 1 d, after pressure application, when the importance of enzyme-substrate interaction is maximum, rheological (G*) and textural (fracture stress) parameters of 300 MPa treated cheeses, both presented high values compared with other HP treatments. This indicated that 300 MPa are the HP conditions which favour interaction between the curd network components.

Time at which pressure treatment was applied had a significant effect on lipolysis. It seems that the application of pressure on d 15 reduced the rate of FFA release (Fig. 1). This slow down of lipolysis may be related to reduced solvent water of the enzyme at this stage of ripening, as free water is the main fraction evaporated in the early stages of ripening Specifically, this kind of water is related to its availability for microbial and chemical processes. In addition, when HP is applied lipid-protein interactions are promoted to some extent depending on the magnitude of HP applied, thus protecting lipid hydrolysis by enzyme action.

Table 3. Free fatty acid concentrations (mg kg^{-1}) found in control and HP-treated ewe milk cheeses at 1 d (a), 15 d (b) and 60 d (c) ripening

Values are means ± SD for $n=4$

a)

	HP treatment†				
	Control	2P1	3P1	4P1	5P1
$C_{4:0}$	1·94 ± 0·15^{a}	2·05 ± 0·15^{a}	2·20 ± 0·14^{a}	1·9 ± 0·02^{a}	1·25 ± 0·16^{b}
$C_{6:0}$	6·45 ± 0·76	6·78 ± 0·11	7·01 ± 0·34	6·3 ± 0·83	6·28 ± 0·05
$C_{8:0}$	4·60 ± 0·32^{b}	4·97 ± 0·15^{b}	9 ± 0·43^{a}	5·53 ± 0·77^{b}	5·03 ± 0·01^{b}
$C_{10:0}$	16·19 ± 1·27^{c}	16·10 ± 0·47^{c}	31·46 ± 0·48^{a}	19·99 ± 2·83^{b}	17·96 ± 0·01^{bc}
$C_{12:0}$	8·63 ± 0·65^{c}	8·37 ± 0·25^{c}	16·64 ± 0·43^{a}	10·57 ± 1·48^{b}	9·78 ± 0·09^{b}
$C_{14:0}$	15·78 ± 1·16^{b}	16·03 ± 0·61^{b}	35·99 ± 2·22^{a}	18·82 ± 2·57^{b}	16·19 ± 0·02^{b}
$C_{16:0}$	46·83 ± 5·58^{b}	61·78 ± 8·10^{b}	109·44 ± 6·47^{a}	51·19 ± 12·36^{b}	48·26 ± 0·02^{b}
$C_{18:0}$	23·32 ± 4·45^{b}	23·17 ± 1·76^{b}	43·95 ± 6·72^{a}	26·83 ± 3·33^{b}	36·33 ± 0·85^{a}
$C_{18:1}$	15·50 ± 3·04^{b}	16·15 ± 0·32^{b}	31·14 ± 1·21^{a}	17·98 ± 2·83^{b}	17·46 ± 0·01^{b}
$C_{18:2}$	7·28 ± 1·58^{b}	10·39 ± 4·69^{ab}	14·63 ± 0·58^{a}	9·51 ± 0·47^{ab}	6·17 ± 0·4^{b}
TOTAL	154·12 ± 11·66^{b}	165·79 ± 12·48^{b}	301·48 ± 4·58^{a}	168·62 ± 27·53^{b}	164·71 ± 1·53^{b}
SCFA	12·96 ± 0·94^{b}	13·79 ± 0·13^{b}	18·37 ± 0·8^{a}	13·73 ± 1·33^{b}	12·56 ± 0·18^{b}
MCFA	40·5 ± 3·01^{c}	40·5 ± 1·31^{c}	85 ± 3·13^{a}	49·37 ± 5·63^{b}	43·93 ± 0·1^{c}
LCFA	95·49 ± 12·07^{b}	111·49 ± 13·8^{b}	197·87 ± 3·91^{a}	105·51 ± 15·52^{b}	108·22 ± 1·01^{b}

b)

	HP treatment†									Effects (P)§		
	Control	2P1	3P1	4P1	5P1	2P15	3P15	4P15	5P15	P	T	P * T
$C_{4:0}$	39·01 ± 3·88^{ab}	13·35 ± 0·89^{d}	34·27 ± 4·25^{bc}	9·97 ± 0·29^{d}	8·33 ± 0·33^{d}	29·42 ± 0·38^{c}	41·88 ± 6·65^{a}	36·18 ± 4·52^{ab}	28·60 ± 2·87^{c}	***	***	***
$C_{6:0}$	25·94 ± 2·98^{a}	11·60 ± 0·71^{d}	23·93 ± 3·07^{ab}	9·46 ± 0·21^{d}	8·28 ± 0·64^{d}	17·45 ± 0·09^{c}	23·79 ± 2·94^{ab}	21·13 ± 2·78^{b}	17·27 ± 1·61^{c}	***	***	***
$C_{8:0}$	16·03 ± 2·49^{a}	8·71 ± 0·67^{d}	16·55 ± 2·59^{a}	8·24 ± 0·21^{d}	7·72 ± 0·44^{d}	10·36 ± 0·14^{cd}	14·61 ± 1·84^{ab}	12·62 ± 2·16^{bc}	10·73 ± 0·99^{cd}	***	**	**
$C_{10:0}$	32·09 ± 2·73^{b}	28·59 ± 1·62^{bc}	37·31 ± 4·74^{a}	26·74 ± 0·76^{c}	26·81 ± 0·88^{c}	23·87 ± 0·04^{c}	28·53 ± 1·97^{bc}	25·8 ± 2·70^{c}	25·96 ± 1·75^{c}	***	***	**
$C_{12:0}$	15·53 ± 0·75^{b}	14·88 ± 0·91^{bc}	17·77 ± 2·09^{a}	13·78 ± 0·37^{bcd}	13·89 ± 0·38^{bcd}	12·28 ± 0·06^{d}	13·99 ± 0·78^{bcd}	13·04 ± 1·40^{cd}	14·13 ± 0·54^{bcd}	***	***	**
$C_{14:0}$	33·92 ± 1·32^{b}	30·19 ± 1·77^{bc}	40·93 ± 6·28^{a}	25·2 ± 0·80^{c}	25·65 ± 0·59^{c}	25·72 ± 0·23^{c}	29·89 ± 1·31^{bc}	28·49 ± 4·03^{bc}	31·49 ± 0·83^{bc}	***	ns	***
$C_{16:0}$	102·10 ± 3·15^{b}	74·18 ± 4·91^{cd}	147·54 ± 28·45^{a}	60·46 ± 1·45^{d}	60·79 ± 2·35^{d}	83·84 ± 4·91^{bcd}	95·07 ± 0·42^{bc}	89·07 ± 14·78^{bc}	98·05 ± 8·71^{bc}	***	ns	***
$C_{18:0}$	32·83 ± 5·26^{b}	36·19 ± 2·43^{b}	30·65 ± 1·91^{b}	30·75 ± 0·71^{b}	32·04 ± 2·36^{b}	32·42 ± 7·35^{b}	34·62 ± 6·6^{b}	33·32 ± 5·37^{b}	71·08 ± 8·36^{a}	***	***	***
$C_{18:1}$	31·51 ± 1·68^{b}	26·2 ± 1·57^{bcd}	38·2 ± 5·56^{a}	22·50 ± 0·74^{d}	22·77 ± 0·66^{d}	23·99 ± 0·86^{cd}	29·16 ± 0·79^{bc}	27·36 ± 4·38^{bcd}	29·33 ± 2·12^{bc}	***	ns	***
$C_{18:2}$	11·97 ± 2·30	13·41 ± 4·07	12·58 ± 1·01	12 ± 0·57	12·93 ± 0·73	9 ± 1·29	11·1 ± 2·07	12·18 ± 2·08	10·04 ± 2·40	ns	**	ns
TOTAL	340·94 ± 21·51^{b}	257·31 ± 14·15^{cd}	399·75 ± 59·79^{a}	219·12 ± 5·43^{d}	219·21 ± 5·68^{d}	268·38 ± 11·55^{cd}	325·93 ± 22·99^{b}	299·21 ± 44·06^{bc}	351·78 ± 12·84^{b}	***	***	***
SCFA	80·98 ± 9·3^{a}	33·66 ± 2·27^{c}	74·75 ± 9·9^{a}	27·67 ± 0·68^{c}	24·32 ± 1·04^{c}	57·24 ± 0·6^{b}	80·28 ± 11·44^{a}	69·93 ± 9·46^{a}	56·61 ± 5·48^{b}	***	***	***
MCFA	81·55 ± 4·8^{b}	73·67 ± 4·31^{bc}	96·02 ± 13·12^{a}	65·72 ± 1·93^{c}	66·35 ± 1·7^{c}	61·88 ± 0·3^{c}	72·42 ± 4·05^{bc}	67·33 ± 8·14^{c}	73·06 ± 1·96^{bc}	*	ns	**
LCFA	178·41 ± 8·38^{b}	149·97 ± 7·86^{b}	228·97 ± 36·82^{a}	125·73 ± 2·97^{b}	128·54 ± 5·17^{b}	149·25 ± 11·89^{b}	147·17 ± 43^{b}	161·94 ± 26·47^{b}	224·58 ± 34·85^{a}	**	ns	***

c)

	Control	HP treatment†							Effects (P)§		
		2P1	3P1	4P1	5P1	2P15	3P15	4P15	5P15	P	T	P * T
$C_{4:0}$	124·01 ± 0·97^{b}	93·77 ± 30·93^{c}	116·06 ± 2·56^{b}	43·70 ± 0·29^{d}	147·28 ± 8·13^{a}	81·12 ± 4·07^{c}	86·70 ± 2·92^{c}	80·65 ± 1·42^{c}	86·4 ± 7·84^{c}	***	***	***
$C_{6:0}$	59·33 ± 0·56^{a}	46·53 ± 12·27^{b}	55·93 ± 1·69^{a}	25·28 ± 0·17^{c}	30·22 ± 1·62^{c}	40·98 ± 1·99^{b}	43·56 ± 1·23^{b}	43·73 ± 0·55^{b}	50·9 ± 4·32^{b}	***	**	***
$C_{8:0}$	29·94 ± 0·35^{bc}	27·89 ± 1·95^{c}	37·1 ± 1·31^{a}	19·96 ± 0·26^{e}	17·50 ± 0·97^{f}	24·24 ± 1·14^{d}	25·42 ± 0·89^{d}	24·05 ± 0·34^{d}	31·71 ± 2·61^{b}	***	ns	***
$C_{10:0}$	63·9 ± 2·65^{cd}	69·73 ± 10·74^{abc}	75·62 ± 3·03^{a}	58·37 ± 0·62^{d}	44·64 ± 2·29^{e}	65·21 ± 3·02^{bcd}	69·61 ± 2·23^{abc}	63·96 ± 1·64^{cd}	74·81 ± 6·46^{ab}	***	***	***
$C_{12:0}$	30·91 ± 1·21^{a}	34·57 ± 6·03^{a}	35·18 ± 1·56^{a}	29·61 ± 0·55^{a}	22·1 ± 1·14^{b}	32·67 ± 1·59^{a}	35·42 ± 1·18^{a}	32·84 ± 0·87^{a}	34·31 ± 2·94^{a}	***	***	***
$C_{14:0}$	76·97 ± 1·99^{ab}	77·03 ± 4·28^{ab}	81·71 ± 3·67^{a}	63·24 ± 1·82^{c}	48·31 ± 2·43^{d}	74·31 ± 4·20^{ab}	80·17 ± 2·87^{a}	73·39 ± 2·14^{ab}	69·22 ± 5·82^{b}	***	***	***
$C_{16:0}$	260·31 ± 18·23^{a}	233·77 ± 68·79^{ab}	243·67 ± 29·41^{a}	200·7 ± 24·1^{abcd}	199·4 ± 19·8^{abcd}	178·71 ± 14·4^{bcd}	219·52 ± 7·85^{abc}	168·12 ± 7·07^{cd}	152·62 ± 10·26^{d}	***	***	ns
$C_{18:0}$	60·7 ± 29·46^{ab}	59·24 ± 6·21^{ab}	57·53 ± 6·26^{ab}	53·58 ± 0·84^{ab}	42·99 ± 3·32^{b}	57·96 ± 5·54^{ab}	64·31 ± 5·69^{ab}	68·56 ± 6·76^{a}	62·02 ± 5·05^{ab}	***	**	ns
$C_{18}1$	59·29 ± 13·18^{b}	61·28 ± 0·41^{b}	90·7 ± 9·35^{a}	50·52 ± 1·52^{bc}	42·72 ± 2·32^{c}	50·43 ± 2·08^{bc}	58·82 ± 4·58^{b}	53·57 ± 0·88^{bc}	61·08 ± 5·93^{b}	***	*	***
$C_{18}2$	42·76 ± 10·78^{b}	44·91 ± 20·66^{b}	40·71 ± 3·55^{b}	62·03 ± 1·49^{a}	18·89 ± 3·35^{c}	39·74 ± 2·31^{b}	39·58 ± 2·33^{b}	38·31 ± 1·38^{b}	38·62 ± 5·96^{b}	ns	ns	***
TOTAL	800·46 ± 19·75^{a}	708·52 ± 42·50^{b}	842·7 ± 62·42^{a}	614·09 ± 16·94^{c}	609·69 ± 40·25^{c}	636·11 ± 28·30^{bc}	723·13 ± 27·37^{b}	647·2 ± 5·32^{bc}	661·4 ± 49·72^{bc}	***	ns	***
SCFA	194·94 ± 36·88^{ab}	168·29 ± 44·5^{abc}	207·48 ± 5·57^{a}	88·94 ± 0·62^{d}	195·01 ± 9·94^{ab}	146·34 ± 7·08^{c}	155·69 ± 4·29^{bc}	148·44 ± 3·32^{c}	168·7 ± 14·76^{abc}	***	ns	***
MCFA	186·93 ± 30·67^{a}	187·25 ± 28·59^{a}	190·12 ± 8·27^{a}	151·23 ± 2·86^{a}	106·88 ± 14·69^{b}	154·41 ± 34·29^{a}	185·2 ± 6·29^{a}	170·19 ± 4·66^{a}	178·34 ± 15·22^{a}	***	*	***
LCFA	472·27 ± 89·71^{a}	399·21 ± 85·8^{abc}	427·08 ± 48·58^{ab}	360·3 ± 33·68^{bc}	304·06 ± 19·81^{c}	326·82 ± 16·62^{bc}	382·24 ± 18·4^{abc}	328·57 ± 11·84^{bc}	314·35 ± 21·14^{c}	***	*	ns

$^{a-f}$ Means in the same row followed by different letters are significantly different ($P \leqslant 0{\cdot}05$)

† Treatments: 2P–5P, cheeses treated at 200, 300, 400 and 500 MPa respectively; P1–P15, pressure applied on the 1st or 15th day of manufacturing

SCFA = $\Sigma(C_{4:0}–C_{8:0})$, MCFA = $\Sigma(C_{10:0}–C_{14:0})$, LCFA = $\Sigma(C_{16:0}–C_{18:2})$, TOTAL = $\Sigma(C_{4:0}–C_{18:2})$

§ Statistical significance: P = high-pressure treatment, T = moment of pressure application; *** $P \leqslant 0{\cdot}001$, ** $P \leqslant 0{\cdot}01$, * $P \leqslant 0{\cdot}1$, ns = not significant

In conclusion, results indicate that pressure of 300 MPa applied on the first day of ripening increased the lipolytic process, which could be caused by the early lysis of cells and better interaction of microbial lipases with the fat. The levels of lipolysis in 3P1-cheeses at 1 d ripening were twice that of the control, which suggested the application of this HP treatment to accelerate lipolysis in ewes' milk cheese. However, pressures $\geqslant$400 MPa decelerated the lipolysis in cheeses, suggesting the use of these treatments to arrest the lipolysis in those cheese varieties where an excessive lipolysis is not desirable as this may lead to a rancidity defect.

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