# High-pressure treatment applied throughout ripening of a goat cheese caused minimal changes on free fatty acids content and oxidation in mature cheese

Francisco Delgado, González-Crespo, Cava, Ramírez

Dairy Sci. & Technol. (2012) 92:237–248
DOI 10.1007/s13594-012-0062-9

ORIGINAL PAPER

# High-pressure treatment applied throughout ripening of a goat cheese caused minimal changes on free fatty acids content and oxidation in mature cheese

**Francisco José Delgado · José González-Crespo · Ramón Cava · Rosario Ramírez**

Received: 6 September 2011 / Revised: 30 January 2012 / Accepted: 30 January 2012 /
Published online: 7 March 2012


**Abstract** High pressure (HP) has rapidly been gaining in importance for the food processing sector due to recent technological developments and increasing consumer demand for minimally processed and healthy foods. However, it is necessary to study the effect of HP on quality parameters of each food product. Changes in free fatty acids (FFA) content and oxidation markers can modify the organoleptic properties of HP-treated cheese. For this reason, the effect of high-pressure treatment on the FFA profile and lipid and protein oxidation of raw milk goat cheese (Ibores PDO cheese) was evaluated. HP treatment at 400 or 600 MPa, at 10 °C, for 7 min was applied at three different times during ripening (1, 30 or 50 days). The differences in FFA concentration detected after HP processing had equilibrated by the end of the ripening process, that is day 60. Finally, a significant increase in lipid oxidation was detected in HP-treated mature cheeses (60 days old) while no differences were found in protein oxidation. Further studies are necessary to understand the relationship between sensory properties and lipid oxidation changes induced by HP treatment of Ibores PDO cheese.

F. J. Delgado (✉) · J. González-Crespo · R. Ramírez
Instituto Tecnológico Agroalimentario (INTAEX) (Technological Agri-food Institute),
Carretera San Vicente s/n, 06071 Badajoz, Spain
e-mail: fjdmartinez@yahoo.es

J. González-Crespo
e-mail: jose.gonzalezcrespo@juntaextremadura.es

R. Ramírez
e-mail: mariarosario.ramirez@juntaextremadura.es

R. Cava
Research Group Tradinnoval, Faculty of Veterinary Science, University of Extremadura, Avda. de la Universidad s/n, 10071 Cáceres, Spain
e-mail: rcava@unex.es

高压处理对山羊奶干酪中游离脂肪酸含量及脂肪氧化作用的影响

**摘要** ：随着消费者对轻度加工食品和健康食品需求量的增加，一些新兴的加工技术引起人们的关注，并在食品工业中广泛应用。 对于高压处理技术在食品中的应用，有必要了解高压处理对食品加工工艺参数和食品质量的影响。 高压处理的干酪其感官特性会因游离脂肪酸含量和氧化指标的改变而受到影响。基于此原因，本文研究了经高压处理后，游离脂肪酸以及脂肪和蛋白质氧化对由生鲜奶制造的Ibores PDO山羊奶干酪品质的影响。在10 °C条件下，对三个不同成熟期(1d、30d和50d)的干酪分别在400MPa和600MPa下处理7min。高压处理后的干酪成熟到60天后测定干酪中游离脂肪酸的变化。高压导致脂肪氧化作用显著地增强，但是不能引起蛋白质的氧化。因此，有必要进一步研究由于高压引起的脂肪氧化与干酪感官特性之间的关系。

**Keywords** Raw goat milk cheese · High pressure · Free fatty acids · Oxidation

**关键词** 山羊奶干酪 · 高压 · 游离脂肪酸 · 氧化

## 1 Introduction

Recent technological developments, combined with a growth in the consumers' demand for high-quality and minimally processed foods, have resulted in a renewed interest in the potential of high pressure (HP) in food processing (Rastogi et al. 2007). HP processing has a lower impact on food properties such as the nutritional value or flavour in comparison to thermal treatment since small molecules such as amino acids or vitamins are only slightly affected by HP (Balci and Wilbey 1999). According to the scientific literature, the HP treatment of cheese has been performed over a wide range of pressure intensities (50–800 MPa) and holding times (from seconds to days) depending on the objectives (Rastogi et al. 2007). However, from a practical viewpoint, HP treatment normally uses medium/high pressures (400–600 MPa) and short holding times (5–10 min), which allow foods to be processed quickly and improve their preservation.

The application of HP can increase the microbial safety of raw milk cheeses which may present some microbial risks (Trujillo et al. 2002). Another application is the use of HP to accelerate the cheese ripening process, which was first described in a patent by Yokoyama et al. (1992). In this work, Cheddar and Parmesan cheeses were HP treated from 5 to 300 MPa for 3 days at 25 °C, and the effect of HP on proteolysis and flavour development was studied. It is reported that HP treatment at 50 MPa for 3 days at 25 °C on 1-day-old cheeses gave rise to an HP-treated cheese similar to a 6-month-old control cheese, so it reduced the time of ripening from 6 months to 3 days. Consequently, a significant reduction of the ripening period of these cheeses was obtained through the use of HP treatment. However, in this patent, ten times more starter bacteria were used, and these results could not be replicated by others (O'Reilly et al. 2000). On the other hand, the ripening process of other types of cheese may be unaffected by HP. For example, lipolysis in Camembert cheese was only slightly affected by HP treatment of up to 500 MPa for 4 h, while in Gouda cheese, the ripening processes were not modified (Kolakowski et al. 1998). More recently, some authors have reported that HP treatment of cheese resulted in a limited production of free fatty acids (FFA) (Saldo et al. 2003) or an arrested cheese ripening

(Wick et al. 2004; Voigt et al. 2010), i.e. there was a decrease or arrest of compounds released into cheese matrix due to the HP treatment. Therefore, this suggests that there are several different factors involved in the evolution and intensity of the ripening changes, e.g. holding time, pressure intensity and the moment of treatment and/or cheese type.

Recent studies carried out in our laboratory (Delgado et al., unpublished data of this research team) indicated an increase in indices of lipid oxidation (thiobarbituric acid reactive species, TBA-RS) over the course of ripening (1, 30, 60 and 90 days) of untreated Ibores cheeses, which can influence the final quality of cheeses by the end of ripening. Changes to this reaction can potentially modify the original aroma profile and may even be associated with the appearance of off flavours. Protein oxidation has also been related to the reduction of protein bio-availability and the formation of toxic compounds (Moreaux and Birlouez-Aragon 1997; Naranjo et al. 1998). Nevertheless, very few studies have evaluated the changes in oxidative markers throughout cheese ripening (Delgado et al. 2009; Fedele and Bergamo 2001), and no studies exist concerning the oxidative changes after HP treatment. With regard to the effect of HP on FFA content in goat cheese, Buffa et al. (2001) studied the FFA profiles of cheeses made from raw, pasteurized or pressure-treated (500 MPa, 15 min, 20 °C) goat milk to assess the effect of milk HP treatment on cheese lipolysis. Juan et al. (2007) analysed the effect of HP treatment (200, 300, 400 or 500 MPa for 10 min) at two stages of ripening (after 1 and 15 days of ripening) of a cheese made from pasteurized ewe milk.

In a parallel study concerning the effect of HP on volatile compounds of Ibores cheese, we reported that HP treatment induced significant changes in the volatile profile of cheeses when subjected to HP treatment at the beginning of ripening (Delgado et al. 2011); HP treatment decreased the relative abundance of the most volatile compounds but enhanced the formation of ketones and hydrocarbons. The use of HP treatment can enhance the microbial safety of Ibores cheese, but it is very important to evaluate the effect of the HP treatment on reactions involved in cheese quality (such as lipolysis and oxidation reactions). It would be advisable to obtain a HP-treated mature cheese (60 days old) with negligible modifications to these reactions. For this reason, the aim of the present work was to study the effect of using HP treatments at 400 or 600 MPa for 7 min at different ripening stages at which the treatment is applied (1, 30 or 50 days) on the evolution of free fatty acids and changes in lipid and protein oxidation in a raw milk goat cheese.

## 2 Materials and methods

### 2.1 Cheese manufacture

Raw goat milk cheeses were manufactured in a Spanish dairy plant which produces "Ibores" PDO: "Berrocales Trujillanos" (Trujillo, Cáceres, Spain). The same batch of Ibores cheese was used for the study. One lyophilized direct-to-vat mesophilic mixed culture (R-704, 50 units; Chr. Hansen, Hørsholm, Denmark), containing *Lactococcus lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis*, was used as starter. Milk was heated to 30±1 °C, and animal rennet was added (Naturen Plus 175, 20–25 mL per 100 L,

CHR Hansen, Hørsholm, Denmark). After ~90 min, the curd was cut into grains (1–2 cm). Cheeses (1.0–1.1 kg) were pressed for 4–5 h, brine salted and ripened (at 8–12 °C and 80% relative humidity).

## 2.2 Sampling and high-pressure treatment

A total of 52 commercial PDO cheeses were selected at three different stages of ripening (days 1, 30 and 50) and treated at 400 or 600 MPa for 7 min at 10 °C. High-pressure processing was conducted at refrigerated temperature to avoid an excessive increase in temperature due to adiabatic heating during HP treatment. Cheeses were vacuum packed in plastic nylon/polyethylene bags (9.3 mL $O_2$ per square metre per 24 h at 0 °C) and processed in a semi-continuous high-pressure unit NC Hyperbaric Wave 6000/55 (55–L, Burgos, Spain). The pressure increase at 400 or 600 MPa needed 2 min 54 s and 3 min 50 s, respectively; the pressure decrease was instantaneous (1 s). Water was used as the pressure-transmitting medium. Cheeses were analysed after HP treatment and at the end of maturation, except cheeses treated with 50 days of maturation, which were ripened vacuum packed until day 60, when they were analysed. These cheeses were maintained vacuum packed in order to simulate real maturation conditions that would be applied in case HP treatment was considered interesting for dairies. Untreated cheeses were used as control ($n$=4) in each stage of ripening. After HP treatment, half of the cheeses treated ($n$=4) were unpacked and followed the normal ripening in the dairy while the other half were analysed ($n$=4). Therefore, at day 1, 12 cheeses were analysed: control ($n$=4), 400 MPa ($n$=4), 600 MPa ($n$=4); at day 30, 12 cheeses were also analysed: control ($n$=4), 400 MPa ($n$=4), 600 MPa ($n$=4); and at day 60, 28 cheeses were analysed: control ($n$=4) and cheeses treated at days 1, 30 and 50 at 400 or 600 MPa ($n$=24; 3 days × 2 treatments × 4 cheeses per batch).

## 2.3 Moisture content and free fatty acids analysis

Moisture content was measured by the gravimetric method by drying cheese samples at 102 °C (IDF 4A/1982). FFA were extracted and analysed according to Delgado et al. (2009). The different fractions were estimated: short-chain fatty acids (SCFA, C4:0–C8:0), medium-chain fatty acids (MCFA, C10:0–C14:0), long-chain fatty acids (LCFA, C15:0–C18:2 n-6) and unsaturated fatty acids (UFA, C18:1 n-9 and C18:2 n-6).

## 2.4 Lipid oxidation

Lipid oxidation analysis was performed using the 2-TBA method of Salih et al. (1987). Two grams of cheese was homogenized with 7.5 mL of perchloric acid (3.86%) and 0.25 mL of BHT (4.2% in ethanol), to minimise the development of oxidative reactions during extraction, and centrifuged at 2,000 rpm for 2 min. The homogenate was filtered and centrifuged at 3,000 rpm for 2 min. After that, aliquots (in duplicate) were taken and mixed with thiobarbituric acid (0.02 M) and heated at 90 °C for 30 min in a hot water bath. After cooling, tubes were centrifuged at 3,000 rpm for 2 min, and the absorbance was measured at 508, 532 and 600 nm. A

standard curve was prepared using different dilutions of a solution of 1,1,3,3-tetraethoxypropane. TBA-RS levels were expressed as milligrams malondialdehyde per kilogram cheese.

### 2.5 Protein oxidation

Protein oxidation was performed by quantification of carbonyl groups formed during incubation with 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl according to the method described by Oliver et al. (1987). Homogenates (1 g of cheese in 10 mL of 0.15 M KCl buffer) were divided into two 0.1-mL aliquots and placed in eppendorfs. Proteins were precipitated in both aliquots with 1 mL 10% TCA and centrifuged at 3,000 rpm for 5 min. One millilitre of 2N HCl was added to one of the eppendorfs to measure protein concentration, and 1 mL of 0.2% DNPH in 2N HCl was added to the other for carbonyl concentration measurement. Both samples were incubated at room temperature for 1 h. Later, samples were again precipitated with 1 mL of 10% TCA and washed with 1 mL ethanol/ethyl acetate (1:1). Finally, 1.5 mL of 6 M guanidine HCl with 20 mM sodium phosphate buffer was added. Carbonyl concentration was measured on the treated sample by measuring DNPH incorporated on the basis of absorption of 21.0 $mM^{-1}$ $cm^{-1}$ at 370 nm for protein hydrazones. Results were expressed as nanomoles of DNPH fixed per milligram of protein. Protein concentration was calculated by spectrophotometry at 280 nm using bovine serum albumin as standard.

### 2.6 Statistical analysis

One-way analysis of variance was performed to evaluate the differences between the control and HP-treated cheeses. When differences were significant, a post hoc Tukey's test was applied to compare mean values. Statistical analysis was performed by SPSS 14.0 software (SPSS Inc., Chicago, IL, USA).

## 3 Results and discussion

### 3.1 Cheeses subjected to HP treatment on day 1

The moisture content of the cheeses subjected to HP treatment on day 1 (Table 1) was not significantly affected immediately after the HP treatment. However, by day 60, the moisture content was significantly modified in HP-treated cheeses with the cheeses treated at 600 MPa having the highest moisture content. These results are in agreement with several studies (Messens et al. 2000; Saldo et al. 2000, 2001; Juan et al. 2008) which found that HP-treated cheeses increased moisture retention in comparison to non-treated control cheeses. These results suggest that HP results in an increase of the water-bingeing capacity in HP-treated cheese and is probably due to alteration of the cheese protein network as a consequence of the HP treatment applied, thus giving rise to a new structure that is more capable of retaining the water in cheese. In general, cheeses with higher moisture content showed a better water-holding capacity. In addition, the pressurization of milk prior to cheese manufacture has been shown to increase the water retention capacity of cheese (Buffa et al. 2003).

**Table 1** Moisture content in control and high-pressure-treated raw goat milk cheeses

| Day of analysis | Control | HP-treated day 1 | | HP-treated day 30 | | HP-treated day 50 | | SEM | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| | | 400 MPa | 600 MPa | 400 MPa | 600 MPa | 400 MPa | 600 MPa | | |
| 2 | 43.7 | 43.7 | 42.87 | | | | | 0.37 | 0.609 |
| 31 | 39.35 | | | 40.83 | 38.96 | | | 0.41 | 0.142 |
| 60 | $35.57^{bc}$ | $39.16^{ab}$ | $40.97^{a}$ | $33.65^{c}$ | $38.33^{abc}$ | $37.5^{abc}$ | $39.17^{ab}$ | 0.56 | 0.002 |

Different letters (a, b, c) in the same row indicate statistically significant differences (Tukey's test, $P<0.05$)

*SEM* Standard error of the mean

The levels of FFA in the control and HP-treated cheeses at day 1 are shown in Tables 2 and 3. Contents of FFA such as butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), pentadecanoic (C15:0), palmitic (C16:0) and linoleic (C18:2 n-6) acids significantly ($P<0.05$) decreased after HP treatment; nevertheless, the total FFA content was similar in HP-treated and control cheeses. These results disagree with those reported by Juan et al. (2007) in pasteurized ewe milk cheese, where capric, lauric and linoleic acids significantly increased after HP treatment at 400 MPa for 10 min at day 1. The FFA reduction reported in HP-treated Ibores cheeses could be linked to the inactivation of certain enzymes (e.g. lipoprotein lipase, microbial lipases) and/or microorganisms. However, as there was only a short time between the application of the HP treatments and the analysis of the cheeses (only 1 day), a longer time may be necessary for changes in the enzymatic activities induced by HP to be translated into changes in the composition of the cheeses. Therefore, modifications caused by HP, e.g. changes in cheese matrix, could have influenced the extraction process itself and resulted in the decrease in the level of certain FFA.

Overall, there were no significant differences in FFA content between both 400- and 600-MPa HP-treated cheeses at the beginning of ripening. According to Juan et al. (2007), pressure intensities (400 or 500 MPa for 10 min) did not have a significant effect on FFA content in 1-day-old ewe cheeses after pressurization.

The significant reduction of the short-chain FFA (butyric (C4:0), caproic (C6:0) and caprylic (C8:0) acids) content after HP treatment (Table 2) could negatively affect cheese flavour as SCFA are known to have low odour thresholds and characteristic tastes (Curioni and Bosset 2002). However, after 60 days of ripening, only three FFA (butyric (C4:0), stearic (C18:0) and linoleic (C18:2 n-6) acids) were significantly reduced ($P<0.05$) by HP treatment. Therefore, the effect of the HP treatment observed at day 1 immediately after treatment may have been reduced during the ripening process. A similar effect was observed for these fatty acids in a study of ewe milk cheeses treated at 400 MPa at day 1 and analysed at day 60 (Juan et al. 2007). However, more individual FFA were affected by the HP treatment used in the latter study in comparison to the present study.

No significant differences ($P>0.05$) were detected at day 1 in the oxidative markers after HP processing at either 400 or 600 MPa (Table 4). However, after 60 days of ripening, HP-treated cheeses showed a significant increase in indices of lipid oxidation. This could be connected to the higher content of linoleic acid in the HP-treated cheeses in comparison to the control cheeses (see Table 3) because lipid

**Table 2** SCFA (C4:0–C8:0) and MCFA (C10:0–C14:0) content (milligrams per kilogram) in control and high-pressure-treated raw goat milk cheeses

| Free fatty acids | Day of analysis | Control | HP-treated day 1 | | HP-treated day 30 | | HP-treated day 50 | | SEM | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 400 MPa | 600 MPa | 400 MPa | 600 MPa | 400 MPa | 600 MPa | | |
| Butyric (C4:0) | 2 | 744.1$^{a}$ | 677.9$^{b}$ | 669.4$^{b}$ | | | | | 11.6 | 0.002 |
| | 31 | 746.3 | | | 734.5 | 723.4 | | | 4.8 | 0.154 |
| | 60 | 834.5$^{a}$ | 781.2$^{ab}$ | 729.4$^{b}$ | 802.7$^{ab}$ | 793.4$^{ab}$ | 785.3$^{ab}$ | 761.2$^{ab}$ | 8.1 | 0.009 |
| Caproic (C6:0) | 2 | 120.7$^{a}$ | 107.9$^{b}$ | 106.3$^{b}$ | | | | | 2.1 | 0.001 |
| | 31 | 124.6$^{a}$ | | | 122.5$^{ab}$ | 118.7$^{b}$ | | | 0.9 | 0.008 |
| | 60 | 138.4 | 131.5 | 122.2 | 130.6 | 130.2 | 130.4 | 127.8 | 1.3 | 0.057 |
| Caprylic (C8:0) | 2 | 43.8$^{a}$ | 35.5$^{b}$ | 35.2$^{b}$ | | | | | 1.3 | 0.001 |
| | 31 | 65.4 | | | 67.7 | 62.0 | | | 1.4 | 0.259 |
| | 60 | 80.0 | 79.1 | 69.5 | 77.8 | 78.4 | 79.6 | 78.3 | 1.4 | 0.492 |
| Capric (C10:0) | 2 | 122.1$^{a}$ | 104.0$^{b}$ | 102.6$^{b}$ | | | | | 3.2 | 0.005 |
| | 31 | 179.1 | | | 201.5 | 182.5 | | | 4.9 | 0.134 |
| | 60 | 226.6 | 231.6 | 203.9 | 228.9 | 229.0 | 234.1 | 226.8 | 3.9 | 0.511 |
| Lauric (C12:0) | 2 | 97.0$^{a}$ | 86.8$^{b}$ | 86.2$^{b}$ | | | | | 1.8 | 0.003 |
| | 31 | 126.4$^{b}$ | | | 142.8$^{a}$ | 131.0$^{ab}$ | | | 2.9 | 0.043 |
| | 60 | 154.5 | 160.0 | 145.2 | 157.2 | 158.5 | 159.6 | 154.8 | 2.1 | 0.589 |
| Myristic (C14:0) | 2 | 156.9 | 145.8 | 143.3 | | | | | 2.6 | 0.054 |
| | 31 | 209.1$^{b}$ | | | 247.1$^{a}$ | 222.3$^{ab}$ | | | 6.0 | 0.011 |
| | 60 | 269.4 | 282.8 | 256.7 | 274.0 | 276.4 | 282.9 | 275.0 | 3.6 | 0.523 |
| SCFA | 60 | 1,052.9$^{a}$ | 991.9$^{ab}$ | 921.1$^{b}$ | 1,011.0$^{ab}$ | 1,002.0$^{ab}$ | 995.3$^{ab}$ | 967.3$^{ab}$ | 10.4 | 0.021 |
| MCFA | 60 | 650.4 | 674.4 | 605.8 | 660.1 | 663.9 | 676.6 | 656.6 | 9.6 | 0.545 |

Different letters (a, b) in the same row indicate statistically significant differences (Tukey's test, $P<0.05$)

*SEM* standard error of the mean, *SCFA* short-chain fatty acids, *MCFA* medium-chain fatty acids

**Table 3** LCFA (C15:0–C18:2 n-6) content (milligrams per kilogram) in control and high-pressure-treated raw goat milk cheeses

| Free fatty acids | Day of analysis | Control | HP-treated day 1 | | HP-treated day 30 | | HP-treated day 50 | | SEM | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 400 MPa | 600 MPa | 400 MPa | 600 MPa | 400 MPa | 600 MPa | | |
| Pentadecanoic (C15:0) | 2 | 85.5$^{a}$ | 79.3$^{b}$ | 78.3$^{b}$ | | | | | 1.1 | 0.002 |
| | 31 | 84.6$^{b}$ | | | 87.1$^{a}$ | 83.9$^{b}$ | | | 0.5 | 0.014 |
| | 60 | 92.2 | 89.6 | 85.8 | 88.3 | 89.7 | 88.7 | 86.3 | 0.6 | 0.102 |
| Palmitic (C16:0) | 2 | 524.5$^{a}$ | 440.1$^{b}$ | 439.4$^{b}$ | | | | | 15.4 | 0.014 |
| | 31 | 625.8$^{b}$ | | | 795.0$^{a}$ | 691.9$^{b}$ | | | 24.5 | 0.003 |
| | 60 | 944.9 | 883.5 | 811.1 | 849.2 | 858.8 | 897.0 | 866.7 | 12.4 | 0.114 |
| Stearic (C18:0) | 2 | 292.3 | 257.8 | 253.9 | | | | | 8.7 | 0.140 |
| | 31 | 298.9$^{c}$ | | | 410.7$^{a}$ | 344.8$^{b}$ | | | 14.9 | 0.001 |
| | 60 | 501.4$^{a}$ | 435.7$^{ab}$ | 412.0$^{b}$ | 412.5$^{b}$ | 416.6$^{b}$ | 447.2$^{ab}$ | 419.5$^{b}$ | 7.9 | 0.010 |
| Oleic (C18:1 n-9) | 2 | 465.0 | 498.2 | 463.2 | | | | | 14.8 | 0.598 |
| | 31 | 619.9$^{b}$ | | | 875.5$^{a}$ | 731.6$^{b}$ | | | 36 | 0.001 |
| | 60 | 906.2 | 995.4 | 998.4 | 958.2 | 947.6 | 1028.1 | 991.6 | 14.5 | 0.374 |
| Linoleic (C18:2 n-6) | 2 | 134.2$^{a}$ | 121.4$^{ab}$ | 119.8$^{b}$ | | | | | 2.6 | 0.026 |
| | 31 | 198.7 | | | 231.0 | 204.5 | | | 6.5 | 0.083 |
| | 60 | 217.7$^{b}$ | 279.6$^{a}$ | 260.1$^{ab}$ | 271.5$^{ab}$ | 276.7$^{a}$ | 278.9$^{a}$ | 269.7$^{ab}$ | 5.6 | 0.017 |
| LCFA | 60 | 2,662.4 | 2,683.9 | 2,567.4 | 2,579.8 | 2,589.4 | 2,739.9 | 2,633.8 | 32.4 | 0.816 |
| UFA | 60 | 1,123.9 | 1,275.0 | 1,258.5 | 1,229.7 | 1,224.3 | 1,307.0 | 1,261.3 | 19.0 | 0.232 |
| UFA/LCFA | 60 | 0.42$^{c}$ | 0.47$^{b}$ | 0.49$^{a}$ | 0.48$^{ab}$ | 0.47$^{b}$ | 0.48$^{ab}$ | 0.48$^{ab}$ | 0.00 | 0.001 |
| Total FFA | 60 | 4,365.7 | 4,350.2 | 4,094.4 | 4,251.0 | 4,255.3 | 4,411.8 | 4,257.6 | 45.8 | 0.653 |

Different letters (a, b, c) in the same row indicate statistically significant differences (Tukey's test, $P<0.05$)

*SEM* standard error of the mean, *LCFA* long-chain fatty acids, *UFA* unsaturated fatty acids, *FFA* free fatty acids

**Table 4** Protein (nanomoles of carbonyls per milligram protein) and lipid oxidation (milligrams of MDA per kilogram) markers in control and high-pressure-treated raw goat milk cheeses

| | Day of analysis | Control | HP-treated day 1 | | HP-treated day 30 | | HP-treated day 50 | | SEM | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 400 MPa | 600 MPa | 400 MPa | 600 MPa | 400 MPa | 600 MPa | | |
| Protein oxidation | 2 | 1.01 | 1.42 | 0.92 | | | | | 0.13 | 0.271 |
| | 31 | 1.25 | | | 1.09 | 1.04 | | | 0.08 | 0.592 |
| | 60 | 1.26 | 1.04 | 1.33 | 1.49 | 1.46 | 1.89 | 1.10 | 0.08 | 0.081 |
| Lipid oxidation | 2 | 0.042 | 0.035 | 0.034 | | | | | 0.00 | 0.123 |
| | 31 | 0.027 | | | 0.029 | 0.030 | | | 0.00 | 0.688 |
| | 60 | $0.027^{b}$ | $0.046^{a}$ | $0.043^{a}$ | $0.036^{ab}$ | $0.036^{ab}$ | $0.047^{a}$ | $0.040^{a}$ | 0.00 | 0.001 |

Different letters (a, b) in the same row indicate statistically significant differences (Tukey's test, $P<0.05$)

*SEM* standard error of the mean

oxidation usually involves the reaction of unsaturated fatty acids with molecular oxygen. Lipid oxidation can result in the formation of off flavours due to the production of fatty acid hydroperoxides which are themselves unstable and can be converted to other volatile flavour compounds such as aldehydes (Serra et al. 2008). Lipid oxidation can also result in the production of other compounds such as ketones, alcohols and hydrocarbons. In a parallel study, we observed that HP treatment increased the relative abundance of ketones when the treatment was applied at the beginning of ripening (Delgado et al. 2011), and therefore, results of that study could be related with the lipid oxidation development of Ibores cheese.

### 3.2 Cheeses subjected to HP treatment on day 30

The moisture content of the cheeses subjected to HP treatment on day 30 was not significantly different in cheeses analysed immediately after pressurization (Table 1). However, after a further 30 days of ripening, post-pressurization (day 60), the lowest moisture content was found in cheeses which had been subjected to 400 MPa.

The FFA concentrations (milligrams per kilogram) detected in the cheeses subjected to HP treatment at day 30 and control cheeses are shown in Tables 2 and 3. HP treatment at 400 MPa significantly increased the levels of FFA in Ibores cheeses immediately after pressurization in contrast to the results observed following HP treatment on day 1 (with the exception of caproic acid which was higher in the control cheeses). In cheeses treated at 600 MPa, the level of lauric (C12:0), myristic (C14:0), palmitic (C16:0) and oleic (C18:1 n-9) acids increased in comparison to the control cheeses, but these increases were not statistically significant. In addition, the FFA content in cheeses treated at 600 MPa was significantly lower than those found in cheeses treated at 400 MPa indicating a significant effect of pressure on the release of FFA.

As previously indicated, HP treatment can cause changes in cheese matrix, thus affecting the extraction of FFA. Nevertheless, the effect of HP on FFA content was different immediately after HP treatment at days 1 and 30 of cheese ripening, suggesting that other factors may be related to the observed changes in FFA. The

higher FFA content observed in cheeses subjected to HP treatment on day 30 could be attributed to a more rapid and efficient interaction of microbial lipases with their substrates due to the lysis produced by HP treatment as reported Juan et al. (2008). The differences in the levels of FFA observed due to the intensity of the pressure applied (400 or 600 MPa) may be a result of a higher enzymatic inactivation at the higher pressure (Seyderhelm et al. 1996). However, further studies are needed to understand all the factors involved in the effect of HP treatment on FFA composition.

The higher FFA content observed in the cheeses treated at 400 MPa at day 30 was balanced 30 days after treatment (day 60), and similar FFA concentrations were found in the control and HP-treated cheeses at day 60. In contrast to the results of this study, Juan et al. (2007) concluded that the use of pressures ≥400 MPa for 10 min reduced the levels of lipolysis in HP-treated ewe cheeses. The differences in the results of the two studies could be explained by a higher holding time of the HP treatment (7 min compared with 10 min) used in the study of Juan et al. (2007) and the different types of milk used for cheese manufacture (goat compared with ewe) and most probably due to the fact that ewe cheeses were manufactured from pasteurized milk, which would have resulted in the inactivation of some lipolytic enzymes such as lipoprotein lipase and microbial lipases.

The levels of lipid and protein oxidation were not changed immediately after HP treatment at day 30 (Table 4). In addition, by day 60 of ripening, there were no differences between the cheeses which had been subjected to HP treatment at day 30 and the control cheeses. Thus, HP-treated cheeses maintained their oxidative state immediately after HP processing at day 30 and after 30 days of ripening post-pressurization (day 60). These results are significant for cheese makers as subjecting cheeses at this stage of ripening would avoid the modification of the cheese oxidation state which is important to maintain its sensory characteristics.

### 3.3 Cheeses subjected to HP treatment on day 50

Cheeses subjected to HP treatment at day 50 were only analysed at the end of ripening, that is day 60. This is an interesting approach because cheeses were stored in vacuum plastic bags until sampling at day 60; thus, cheese producers would not have to vacuum-pack the cheeses a second time before sale. However, there was not a suitable control cheese, i.e. untreated cheese vacuum packed at day 50. In any case, the moisture content of the cheeses treated at day 50 and analysed at day 60 was slightly higher in the HP-treated cheeses than in the control cheeses (Table 1). As previously discussed, in general, HP treatment resulted in better water retention in pressurized cheeses. Regarding the effect of HP treatment at day 50 on the content of FFA (Tables 2 and 3), there were slight differences between the HP-treated and the control cheeses. Only stearic (C18:0) and linoleic (C18:2 n-6) acid concentrations were significantly changed in HP-treated cheeses (Table 3). A similar trend was observed in cheeses treated at days 1 and 30 when they were analysed at the end of ripening (day 60). Therefore, it is possible that pressurization of Ibores cheese at any stage of ripening (days 1, 30 or 60) would result in minimum changes in the FFA profile of the mature cheese (day 60).

Protein and lipid oxidation indices in the cheeses subjected to HP treatment at day 50 and analysed at day 60 are presented in Table 4. Protein oxidation was not

significantly affected by HP treatment, but significant differences were obtained for lipid oxidation. Higher TBA-RS values were detected in the HP-treated cheeses at days 1 and 50. Despite the results reported in this study, the levels of lipid oxidation detected in this study are lower than values reported for other types of cheeses (Balestrieri et al. 2002; Delgado et al. 2009), and, in a parallel study of three dairies which produce PDO Ibores cheese, similar levels of TBA-RS (~0.05 mg MDA per kilogram) were found to those reported for the HP-treated cheeses of this study. Regardless, the development of indices of lipid oxidation should be taken into account when considering the application of HP processing of cheeses with high initial levels of oxidation, such as in pasteurized milk cheeses, due to the pro-oxidant effect of the heat treatment.

## 4 Conclusions

The differences detected in the FFA concentration after HP processing at days 1 and 30 had equilibrated within the cheeses by the end of the ripening process, that is day 60. Only cheeses subjected to HP treatment and analysed at day 30 showed a variable effect which was dependent on the pressure applied (400 or 600 MPa). Regarding the oxidative markers, protein oxidation was not significantly changed by HP treatment, but lipid oxidation showed a significant increase in cheeses treated at days 1 and 50, analysed at the end of ripening (day 60). Further studies about the effect of HP treatment on lipid oxidation of cheese and the relationship with sensory properties are necessary to evaluate the sensory impact of these changes.

**Acknowledgements** This work has been financed by the European Regional Development Fund (ERDF). Francisco José Delgado thanks Junta de Extremadura for the grant received for this research (DOE 10.10.07). R. Ramírez thanks INIA and ERDF for her hiring (BOE, 10.01.07). We thank the PDO dairy "Berrocales Trujillanos" for providing the cheeses.

## References

Balci AT, Wilbey RA (1999) High pressure processing of milk: the first 100 years in the development of a new technology. Int Dairy J 52:149–155

Balestrieri M, Spagnuolo MS, Cigliano L, Storti G, Ferrara L, Abrescia P, Fedele E (2002) Evaluation of oxidative damage in mozzarella cheese produced from bovine or water buffalo milk. Food Chem 77:293–299

Buffa MN, Guamis B, Pavia M, Trujillo AJ (2001) Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk. Int Dairy J 11:175–179

Buffa M, Guamis B, Saldo J, Trujillo AJ (2003) Changes in water binding during ripening of cheeses made from raw, pasteurized or high-pressure-treated goat milk. Lait 83:89–86

Curioni PMG, Bosset JO (2002) Key odorants in various cheese types as determined by gas chromatography–olfactometry. Int Dairy J 12:959–984

Delgado FJ, González-Crespo J, Ladero L, Cava R, Ramírez R (2009) Free fatty acids and oxidative changes of a Spanish soft cheese (PDO "Torta del Casar") during ripening. Int J Food Sci Tech 44:1721–1728

Delgado FJ, González-Crespo J, Cava R, Ramírez R (2011) Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic high pressure at different stages of maturation. Int Dairy J 21:135–141

Fedele E, Bergamo P (2001) Protein and lipid oxidative stresses during cheese manufacture. J Food Sci 66:932–935

IDF (1982) Cheese and processed cheese: moisture determination, determination of the total solids content (Standard 4A) International Dairy Federation, Brussels
Juan B, Ferragut V, Buffa M, Guamis B, Trujillo AJ (2007) Effects of high-pressure treatment on free fatty acids release during ripening of ewes' milk cheese. J Dairy Res 74:438–445
Juan B, Ferragut V, Guamis B, Trujillo AJ (2008) The effect of high-pressure treatment at 300 MPa on ripening of ewes' milk cheese. Int Dairy J 18:129–138
Kolakowski P, Reps A, Babuchowski A (1998) Characteristics of pressure ripened cheeses. Pol J Food Nutr Sci 48:473–483
Messens W, Van de Walle D, Arevalo J, Dewettinck K, Huyghebaert A (2000) Rheological properties of high-pressure-treated gouda cheese. Int Dairy J 10:359–367
Moreaux V, Birlouez-Aragon I (1997) Degradation of tryptophan in heated αlactoglobulin-lactose mixtures is associated with intense Maillard reaction. J Agric Food Chem 45:1905–1910
Naranjo GB, Malec LS, Vigo MS (1998) Reducing sugars effect on available lysine loss of casein by moderate heat treatment. Food Chem 62:309–313
O'Reilly CE, O'Connor PM, Murphy PM, Kelly AL, Beresford TP (2000) The effect of exposure to pressure of 50 MPa on Cheddar cheese ripening. Innov Food Sci Emerg Tech 1:109–117
Oliver CN, Ahn BW, Moerman EJ, Goldstein S, Satadtman ER (1987) Aged-related changes in oxidized proteins. J Biol Chem 262:5488–5491
Rastogi NK, Raghavarao KSMS, Balasubramaniam VM, Niranjan K, Knorr D (2007) Opportunities and challenges in high pressure processing of foods. Cri Rev Food Sci Nutr 47:69–112
Saldo J, Sendra E, Guamis B (2000) High hydrostatic pressure for accelerating ripening of goat's milk cheese: proteolysis and texture. J Food Sci 65:636–640
Saldo J, Sendra E, Guamis B (2001) Hard cheese structure after a high hydrostatic pressure treatment at 50 MPa for 72 h applied to cheese after brining. Lait 81:625–635
Saldo J, Fernández A, Sendra E, Butz P, Tauscher B, Guamis B (2003) High pressure treatment decelerates the lipolysis in a caprine cheese. Food Res Int 36:1061–1068
Salih AM, Smith DM, Price JF, Dawson LE (1987) Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poultry Sci 66:1483–1489
Serra M, Trujillo AJ, Pereda J, Guamis B, Ferragut V (2008) Quantification of lipolysis and lipid oxidation during cold storage of yogurts produced from milk treated by ultra-high pressure homogenization. J Food Engin 89:99–104
Seyderhelm I, Boguslawski S, Michaelis G, Knorr D (1996) Pressure induced inactivation of selected food enzymes. J Food Sci 61:308–310
Trujillo AJ, Capellas M, Saldo J, Gervilla R, Guamis G (2002) Applications of high-hydrostatic pressure on milk and dairy products: a review. Innov Food Sci Emerg Tech 3:295–307
Voigt DD, Chevalier F, Qian MC, Kelly AL (2010) Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. Innov Food Sci Emerg Tech 11:68–77
Wick C, Nienaber U, Anggraeni O, Shellhammer TH, Courtney PD (2004) Texture, proteolysis and viable lactic acid bacteria in commercial Cheddar cheeses treated with high pressure. J Dairy Res 71:107–115
Yokoyama H, Sawamura N, Motobayashi N (1992) Method for accelerating cheese ripening, European patent application EP 0 469857 A1