

3.5 COLOUR CHANGES DURING RIPENING OF HIGH PRESSURE TREATED HARD CAPRINE CHEESE (2002)

J. Saldo, E. Sendra, B. Guamis. *High Pressure Research* 22 (3-4) 659-663

COLOUR CHANGES DURING RIPENING OF HIGH PRESSURE TREATED HARD CAPRINE CHEESE

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(Received 17 July 2001; In final form 3 October 2001)

High hydrostatic pressure (HHP) treatment of cheese intended to accelerate ripening. Along with increased proteolysis, some other parameters were affected, colour being one of them. Right after HHP and at the end of ripening time, Hunterlab colour parameters were very similar in both control and cheese treated at 400 MPa, but during ripening they evolved in a different way. HHP-treated cheese had lower lightness and higher chroma values than control cheese and both characteristics were unexpectedly associated to higher moisture values. Those differences are attributed to changes in cheese microstructure.

Keywords: colour, goat cheese, ripening, high pressure

INTRODUCTION

Garrotxa cheese is a caprine milk cheese from Catalunya (Spain). Produced from mixed coagulation curds (enzymatic-lactic), its typical ripening time is of 6-8 weeks. The cheese weighs 1 kg and is cylindrical in shape, with rounded edges. The rind is velvety, with a grey-blue colour due to the growth of *Penicillium glaucum* at the surface. This cheese has a firm, yet creamy, ivory-white interior. Its flavour is slightly sour, fatty on the palate and with a hazelnut taste.

High pressure (HHP) treatment at the beginning of cheese ripening has been proved as a successful technique to accelerate cheese ripening. This treatment leads to increased proteolysis, higher moisture content and higher pH [12].

Changes in colour in Garrotxa cheese during regular ripening have not been studied. The same changes in HHP-treated cheese have not been studied, either. The aim of the present study is to enlighten this topic.

MATERIALS AND METHODS

Two batches of cheese were made from 300 L of pasteurised milk each in the Pilot Plant of Food Technology, at the UAB. The cheese making technology was described in Saldo et al. [12]. After brining, cheeses were separated into two groups, one treated at 400 MPa for 5 min (HHP) and acting the other as control. Cheeses were ripened at 14 °C and 83% RH and one piece of cheese of each group was sampled at 3, 7, 14, 21, 30, 45, and 60 days.

Changes in colour were evaluated using a Miniscan™ XE colorimeter (Hunter Associates Laboratory, Reston, VA, USA) with a Fcw illuminant and observer at 10°. The cartesian coordinates of L ; a ; b were converted into polar coordinates by calculation of the longitude of

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the vector in the *a-b* plane (namely Chroma) and the angle of the vector (Hue angle) according with the guidelines of Little [6].

Moisture was evaluated in triplicate by means of the oven drying method [4].

ANOVA and Principal Component analysis were performed using Statistica v5.0 (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Variation in colour among different cheese types depend upon cheese manufacture techniques, and it is mainly affected by qualitative properties of the fat phase [11]. Additional minor sources of variation can explain colour changes in a particular cheese type. Those variations include total solids content as well as other changes produced along cheese ripening.

Table 1. Changes in Hunterlab colour parameters during ripening of control and high hydrostatic pressure (HHP) treated cheese. Two-way ANOVA (ripening time and pressure treatment) was applied to data, and values with different superscript letter are significantly different ($P < 0.05$, HSD test).

	<i>L</i>		<i>a</i>		<i>b</i>		Chroma		Hue angle	
	Ctrl	HHP	Ctrl	HHP	Ctrl	HHP	Ctrl	HHP	Ctrl	HHP
3 days	93.6 ^d	92.5 ^c	-0.84 ^b	-0.94 ^a	8.56 ^c	9.62 ^{d,e}	8.61 ^c	9.67 ^{d,e}	-84.4 ^e	-84.2 ^e
7 days	94.0 ^d	91.6 ^{a,b}	-0.64 ^{d,e}	-0.90 ^a	7.82 ^a	9.44 ^{d,e}	7.85 ^a	9.48 ^{d,e}	-85.3 ^d	-84.5 ^e
14 days	93.4 ^d	92.3 ^c	-0.60 ^{d,e}	-0.69 ^d	8.21 ^b	9.46 ^{d,e}	8.23 ^b	9.48 ^{d,e}	-85.7 ^c	-85.9 ^c
21 days	93.7 ^d	92.6 ^c	-0.60 ^{d,e}	-0.78 ^c	7.86 ^a	9.24 ^d	7.89 ^a	9.27 ^d	-85.7 ^c	-85.3 ^d
30 days	93.4 ^d	92.2 ^c	-0.42 ^h	-0.63 ^{d,e}	7.88 ^a	9.29 ^d	7.89 ^a	9.32 ^d	-87.0 ^b	-86.2 ^c
45 days	92.0 ^{b,c}	92.2 ^c	-0.48 ^g	-0.66 ^d	8.74 ^c	9.34 ^{d,e}	8.76 ^c	9.37 ^{d,e}	-86.8 ^b	-85.9 ^c
60 days	91.4 ^{a,b}	91.3 ^a	-0.40 ^h	-0.55 ^f	9.22 ^a	10.36 ^f	9.23 ^a	10.38 ^f	-87.5 ^a	-86.9 ^b

The decrease in cheese lightness (*L*-values) during ripening was associated with the concentration of cheese components, as occurs in Emmental [10] and Provolone cheeses [9]. The evolution in this parameter was regular in control cheese, but in HHP-treated cheese there is a plateau for the values corresponding to mid-matured samples (Table 1). *L*-values decreased dramatically the days after the treatment to evolve later to values similar to those of control cheese. Those changes due to HHP treatment cannot be attributed to any decrease in moisture, as moisture content was higher in HHP-treated cheese (Table 2), but to a modification of cheese microstructure [13]. The decrease in lightness induced by HHP was noticed on the treatment of immature Mozzarella [5], producing a change comparable with to that of the variation produced by the ripening

and was also related to structural changes induced by HHP.

Table 2. Moisture content during ripening of control cheese and cheese treated at 400 MPa. Two-way ANOVA (ripening time and pressure treatment) was applied to data, and values with different superscript letter are different ($P < 0.05$, HSD test).

	Control	HHP
3 days	48.8% ^a	49.7% ^b
7 days	47.7% ^a	49.8% ^b
14 days	44.5% ^c	48.2% ^a
21 days	43.9% ^c	48.1% ^a
30 days	40.4% ^d	45.9% ^e
45 days	35.9% ^e	42.6% ^f
60 days	33.1% ^g	37.8% ^h

During ripening there was a change in colour to a more yellow-orange colour by increases in *a*- and *b*-values (Table 1). A similar evolution has been reported during ripening of Garrotxa [1] and Emmental [10] cheeses. Colour changes were best depicted by changes in chroma and hue angle.

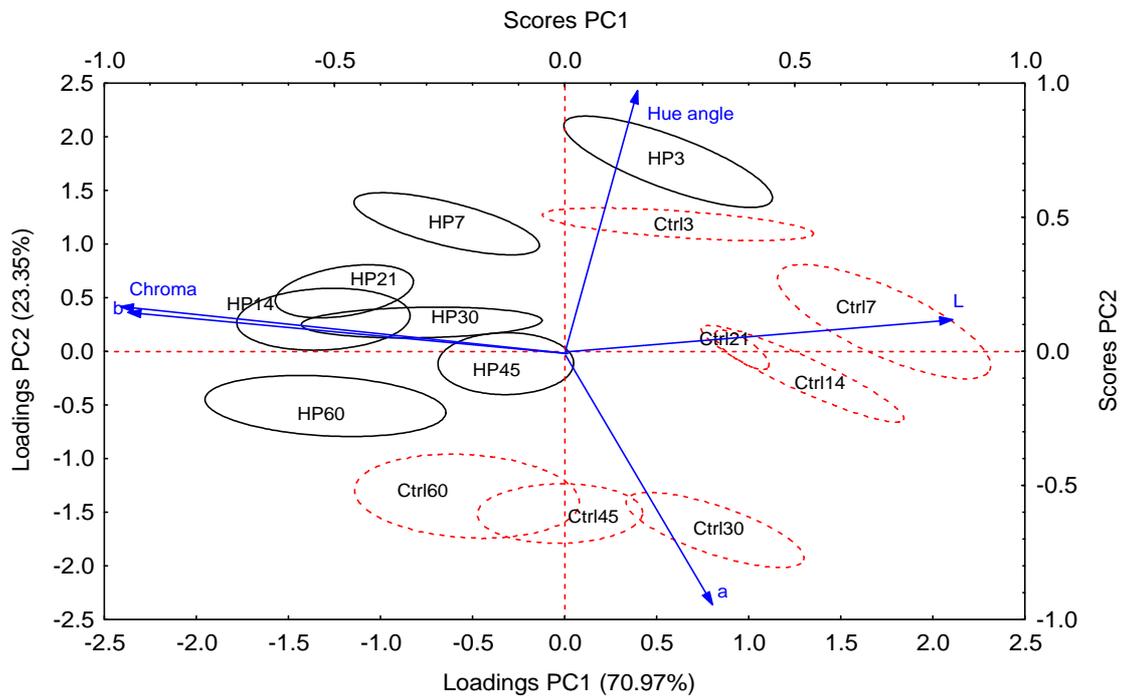


Figure 1. Loading Vectors of colour variables and score plot of measures obtained from cheeses treated by HHP (400 MPa, 5 min) and control. Cheeses were ripened for 3, 7, 14, 21, 30, 45 and 60 days.

From the principal component analysis of colour measurements, two new coordinates were found (PC1 and PC2), explaining about 95% of the variability associated to the samples. In this principal component space each measurement can be drawn, being each sample represented by an ellipse in Figure 1. The size of each contour corresponds to the dispersion of the repeated measures of each sample. PC1 values decreased during ripening, being the mature samples on the left of the score plot. PC2 allowed discrimination between treatments, having untreated cheeses higher PC2 values. HHP samples appear at the bottom of the score plot. The evolution of cheese colour is depicted in Figure 1 by an evolution from top to bottom, the samples of untreated cheese occupying the right part of the picture and those of HHP-treated cheese the left.

The HHP treatment produced a slight change in Hue angle but a clear decrease in *L*-values and an increase in Chroma that was kept during most of the ripening time. A similar effect of HHP on cheese colour was produced by 50 MPa, 72-h treatment [13]. By day 60, control and treated cheese had almost the same colour values.

High pressure affects non-covalent bonds, and in literature reviews on HHP application on foods it is frequent to read that colour is not affected by pressurisation [3, 14]. Since the effect of HHP on pigments can be of minor importance, some other changes can occur, and they do occur in cheese. Cheese structure is defined by the hydrophobic interactions between caseins, being one of the non-covalent interactions broken by HHP [7]. The new organisation of cheese components produces a new structure of different rheological [8] and colour characteristics. The relationships between HHP, microstructure and colour have already been studied in long treatments at moderate pressure (50 MPa for 72 h) [13].

Capellas [2] studied the effect of HHP on fresh caprine cheese. The *b*-parameter was the only colour parameter different between control and cheese treated at 500 MPa which tended to be a bit more yellow. Johnston and Darcy [5] found significant differences in the *b*-parameter in HHP-treated Mozzarella cheese.

In the present study not only the *b*-parameter, but all the HunterLab parameters were changed by the HHP treatment. *L* decreased, whereas *a*- and *b*-parameters increased significantly ($p < 0.05$).

HHP cheeses had higher moisture than control ones, in consequence merely moisture loss and concentration of cheese components can not explain their increase in Chroma. They should be related to previously reported microstructure changes after HHP processing [5, 13] or other unknown mechanisms.

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3.6 CHANGES IN WATER BINDING IN HIGH-PRESSURE TREATED CHEESE, MEASURED BY TGA (THERMOGRAVIMETRICAL ANALYSIS) (2002) J. Saldo, E. Sendra, B. Guamis. *Innovative Food Science and Emerging Technologies (en prensa)*

Changes in water binding in high-pressure treated cheese, measured by TGA (Thermogravimetric analysis)

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Abstract

Garrotxa cheese was pressurised at 400MPa for 5 min to shorten ripening accordingly to previous studies. Moisture content was followed along ripening. Moisture in cheese was divided into free and bound water according to the weight loss rate during drying in a TGA oven. High pressure treated cheese to shorten ripening time retained more moisture than control cheese. Free water remained unaffected by the pressure treatment, whereas bound water was higher in treated cheese during ripening. Free water content was linked with a_w .

Keywords: free water, bound water, high pressure, cheese, thermal analysis

Introduction

High pressure treatments (HPT) have been applied to cheese to extend shelf life of fresh cheese (Capellas, Mor-Mur, Sendra, Pla & Guamis, 1996), to accelerate cheese brining (Messens, Dewettinck & Huyghebaert, 1999; Pavia, Trujillo, Guamis & Ferragut, 2000), and to control enzymatic proteolysis (Kolakowski, Reps & Babuchowski, 1998; O'Reilly, O'Connor, Murphy, Kelly & Beresford, 2000). Some reduction in water losses during cheese ripening of HPT-cheese have been reported in early ripening stages (Saldo, Sendra & Guamis, 2000).

Historically, scientists have investigated water due to its universality and importance in the ultimate quality of foods. Some early work used prescribed methodology to determine free and bound water. Accurate definition of these terms is dependent on the methodology used to determine free water. The amount of water removed depends on which conditions were used to press out the free water. The water that was left was considered as bound water. Many problems arise from these unclear considerations. Recently, modern methodologies have been developed for water analysis in different foods. Thermogravimetry (TG) was applied to Grana Padano cheese by de Angelis Curtis et al. (1999) and to Cabrales cheese by Moro García et al. (1993), Differential Scanning Calorimetry (DSC) was applied to Mozzarella cheese by McMahon et al. (1999) and to milk protein solutions by le Dean et al. (2001) who also applied Nuclear Magnetic Resonance (NMR) techniques. DSC techniques were based on the measurement of free water amount by calculation of heat of ice melting on a sample previously freeze at -40 °C. Low resolution NMR results can be explained by assigning different relaxation rates to protons of different water fractions.

The aim of this study was to determine water distribution in HPT-cheese. Water activity assists in explaining the ability of water to take part in biological and chemical reactions. This work intends to understand differences in water losses and retention in cheese as an effect of HPT by assessing changes in free and bound water.

Materials and Methods

Cheese making and high-pressure treatment

Two batches of cheese were manufactured in the pilot plant of Universitat Autònoma de Barcelona with goat milk as described elsewhere (Saldo et al., 2000) producing cheese pieces of about 1.5 kg. The cheese was produced from pasteurized goat's milk by enzymatic coagulation (0.07 mL/L calf rennet Renifor-15/E from Lamirsa, Terrassa, Spain, containing 780 mg/L active chymosin) and using an homofermentative starter. Curd were pressed and salted in brine (19% NaCl wt/vol, 14 °C, 5 h).

The day after cheese-making cheeses were vacuum packaged and divided into two groups. One group was exposed to high pressure (400 MPa for 5 min at 14 °C) using a ACB HP Horizontal 350 L equipment at España S.A. facilities in Olot (Spain) while the other one remained at 0.1 MPa and act as a control. Right after the HPT packaging material was removed and cheeses were brought back to the ripening chamber (14 °C, 83% RH).

Sampling

Cheeses were analyzed for moisture content, water activity, and water binding at days 3, 7, 14, 21, 30, 45 and 60. From two whole cheeses, the rind was removed and cheese was grated and mixed to obtain one average sample for the entire cheese piece.

TGA instrumentation

The thermogravimetric analyses were performed on a TGA/SDTA851^o thermobalance (Mettler-Toledo GmdH Analytical, Switzerland). Grated cheese samples, weighting about 20 mg each, were placed in the thermobalance alumina sample pan and heated over the temperature range 25-650 °C, at a scanning rate of 5 °C min⁻¹. The analysis was done in duplicate for each of the samples in the study.

Water activity measurements

Water activity was measured in duplicate using Novasina Thermoconstanter TH 200 (Novasina, Switzerland) at 20 °C.

Composition

Moisture content was settled by an oven drying method at 102 °C and water-soluble nitrogen (WSN) was fractionated and quantified by Kjeldahl semi-micro method. Salt content of cheeses was estimated by a potentiometric method using a Chloride Analyser mod. 926 (Sherwood Scientific Ltd. United Kingdom). All composition determinations were done according to Polychroniadou & Ardö (1999).

Statistical analysis

The experiment was done in duplicate, and from each cheese sample, every parameter was analysed in duplicate (moisture content in triplicate). ANOVA analysis was done by means a repeated measures design. Significance of differences was evaluated by LSD test and the significance set at $p < 0.05$. Statistical analysis were done at the "Servei d'Estadística de la UAB" using SAS v6.

Discussion

Examination of the thermogravimetric curves, and their first derivatives shows 3 weight loss steps, numbered from ΔW_1 to ΔW_3 (Fig. 1). The presence of water over the range 25-200 °C has been confirmed by de Angelis Curtis et al. (1999) by means of IR analysis corresponding to

ΔW_1 and ΔW_2 . The anhydrous sample decomposes thermally and the release of their pyrolysis products produces the ΔW_3 weight loss step.

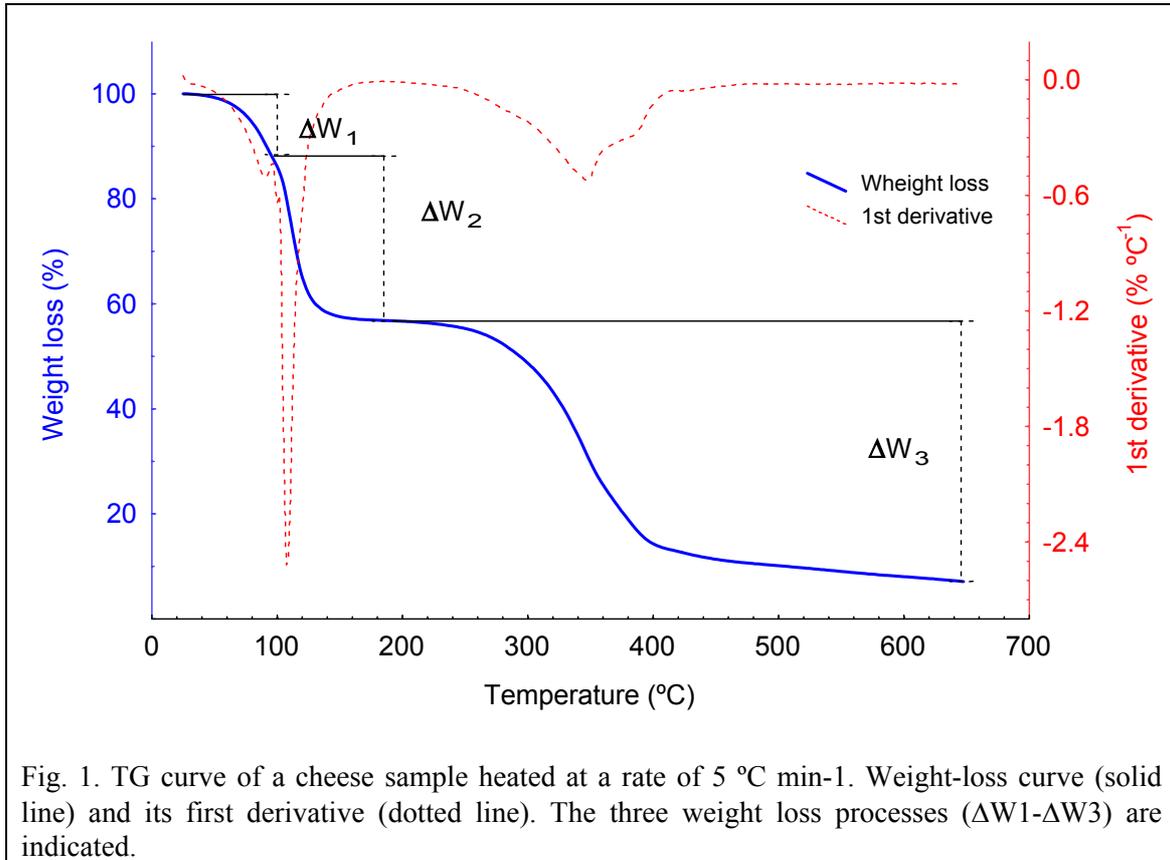


Fig. 1. TG curve of a cheese sample heated at a rate of 5 °C min⁻¹. Weight-loss curve (solid line) and its first derivative (dotted line). The three weight loss processes (ΔW_1 - ΔW_3) are indicated.

The first slight thermogravimetric step (ΔW_1) corresponds to free water that is the water bound with less energy to the matrix. The more strongly linked water to the matrix corresponds to the bound water, and the step ΔW_2 is assigned to this type of water. Water loss occurs through those two partially overlapping processes during TGA. It is possible to distinguish the two processes and assign a limit between them with the aid of the first derivative of the weight loss curve. A local maximum points to the inflexion point, dividing the moisture loss into two parts.

The difference between free and bound water is not straight. In complex systems, such as cheese, there exist different degrees of water binding. Free water is found far from the non-aqueous components, being predominant the water-water hydrogen bonds. When the sample is heated and free water is released, the equilibrium between the different sorts of water changes and the limit between free and bound water become diffused. In preliminary trials, different heating rates (from 1 °C min⁻¹ to 10 °C min⁻¹) were assayed. For the slowest heating rate the water was released without showing any inflexion point in the weigh loss as the multilayer water was released slowly (data not shown). When the heating rate was too high the bound water was not accurately measured because at the inflexion point not all the free water had been already released. So, the heating rate of 5 °C min⁻¹ was chosen as an agreement and the division between both types of water was standardized at 94 °C.

There exists a great agreement between the measures of water content obtained from the desiccation in an oven at 104 °C and those obtained by TGA summing ΔW_1 and ΔW_2 . The correlation between both methods was excellent ($R^2=0.945$), producing equivalent results by measuring moisture content in cheese by TGA or by oven drying. TGA has the advantage of the more rapid results, but the measurements must be done individually while in the oven is possible to do the measurements in parallel for a large number of samples. Moro García et al.

(1993) working on Cabrales cheese found a similar agreement between the moisture content measured by TGA and by conventional oven-drying.

The migration-evaporation process through the cheese rind during the ripening process produces a weigh-loss. The water content reduction was higher in control cheese compared with HPT-cheese. In control cheese, the weight loss during the 2 months ripening process was 30%, although in HPT it was only 25%.

	a_w	Moisture	Free water	Bound water	Salt	WSN
Ripening time	-0.928 ***	-0.905 ***	-0.801 ***	-0.530	0.892 ***	0.950 ***
Treatment	-0.042	-0.381	-0.032	-0.605 *	-0.099	-0.252
a_w	1.000	0.881 ***	0.779 ***	0.531	-0.748 **	-0.822 ***
Moisture		1.000	0.786 ***	0.722 **	-0.736 **	-0.743 **
Free water			1.000	0.208	-0.767 **	-0.727 **
Bound water				1.000	-0.259	-0.333
Salt					1.000	0.920 ***
WSN						1.000

Table 1. Correlation between parameters and measures variables. Marked correlations are significant at * p<0.05, ** p<0.01, and *** p<0.001.

Bound water was not different between treatments, and even the significance of the effect of time on this fraction of water content was scarce. Free water content during cheese ripening was affected by the HPT applied to cheese and correlates significantly with cheese moisture (Table 1). A similar behavior for total and bound water was described by de Angelis Curtis et al. (1999) in a study on Grana Padano cheese. Bound water was affected mainly by solutes present in cheese, namely salts and products of proteolysis and lipolysis. Lipolysis and proteolysis are much more important in long ripened Italian cheeses than in Garrotxa cheese. In the present study the salt content and the WSN produced a decrease in the free water amount (Table 1), but the effect on bound water not significant. In a report on Mozzarella cheese (McMahon, Fife & Oberg, 1999) found that bound water, measured as non-freezable at -40 °C, remained constant during storage. This would mean that only minor changes in bound water would be expected anyway. Bound water was affected by free water through a series of kinetic and thermodynamic equilibria, and thus decreased during ripening.

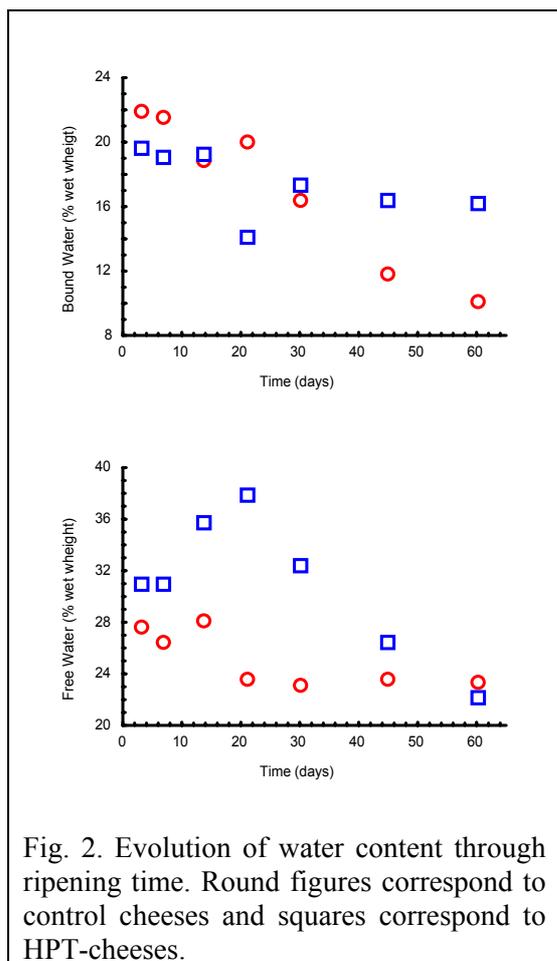


Fig. 2. Evolution of water content through ripening time. Round figures correspond to control cheeses and squares correspond to HPT-cheeses.

Water activity decreased from 0.999 in freshly salted curds to about 0.96 in two-month ripened cheese. This decrease in a_w correlated with the decrease in moisture of cheeses (Table 1). Water activity is a measurement of water available in food for microbial and chemical processes and it is related to free water concept. Free water content correlated significantly with a_w , while bound water content did not show correlation with a_w (Table 1). The decrease in free water caused by WSN and salt was also reflected in a_w .

The HPT applied to cheese is responsible for the changes in water distribution. The total water moisture was reduced during the ripening process being this change noticeably mostly in the free water fraction, which keeps higher in HPT-cheeses (Fig. 2). Johnston & Darcy (2000) found changes in Mozzarella cheese microstructure as a result of HPT at 200 MPa, being water entrapped in interstitial spaces strongly bound and not expressible by centrifugation. This internal redistribution of moisture can explain the diminish on migration-evaporation of water in HPT-cheese. This free water, just entrapped, was released during heating in TGA oven from the grated cheese.

Summary

High pressure treatment of cheese at 400 MPa produced changes in the internal distribution of water. Those changes were accompanied by a higher moisture content, and thus by a decrease in weight loss during ripening. Bound water did not change as a result of treatment, whereas free water was affected significantly by the HPT applied. The higher content of free water increased the output of the cheese-making process, but caused an increase in a_w . The higher a_w could lead to an enhanced enzymatic and microbial activity in cheese.

Acknowledgements:

We would acknowledge the use of the España S.A. high pressure facilities in Olot (Spain). Author J.S. has benefited from a doctoral grant given by the Catalan Commission for Universities and Research.

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3.7 ANALYSIS OF THE VOLATILE FRACTION OF HIGH PRESSURE TREATED CAPRINE CHEESE BY SIMULTANEOUS DISTILLATION-EXTRACTION AND GAS CHROMATOGRAPHY (2002) J. Saldo, A. Fernández, E. Sendra, P. Butz, B. Tauscher, B. Guamis. *European Food Research and Technology* (enviado)

Analysis of the volatile fraction of high pressure treated caprine cheese by simultaneous distillation-extraction and gas chromatography

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Abstract

Garrotxa cheese, a cheese made of goat's milk, typical of the Catalan region, has been high pressure-treated (400 MPa, 5 min, 14 °C) to accelerate the proteolysis rate. Humidity and content of non-casein nitrogen and non-protein were higher on pressurised cheese, but bacterial counts were significantly reduced after pressure treatment. Besides, the volatile fraction of Garrotxa cheese was studied on treated and untreated samples at two ripening stages by SDE extraction with dichloromethane, and identified with GC-MS. Pressure treatment at 400 MPa for 5 min decelerated the lipolysis, having treated cheese lower amount of free fatty acids, and in general less volatile compounds showing a tendency to reduce differences during ripening. Pressure treatment has not produced the release of new volatile compounds in Garrotxa cheese.

Keywords: high pressure, goat's cheese, SDE, volatile compounds, fatty acids

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Introduction

Caprine cheese is highly appreciated by consumers since it has a strong typical flavour. Therefore its marketability is rapidly improving. For example, in France the consumption of goat cheese increased 35% on ten years, being the groups more prone to consume this kind of cheese those who live in the area of production (the south of France) and the people with high incomes in urban areas [1]. Goat cheese is one of the most costly cheeses which makes it a high added value product.

Garrotxa cheese is the mostly consumed goat's cheese in Catalunya (Spain). It is made from pasteurised goat's milk by enzymatic curdling. This cheese has a white interior with a creamy texture, and it is surrounded by a grey, natural mould rind. It has a mild flavour with a light acidity. Typical ripening time is 6 to 8 weeks for 1 kg pieces.

The application of moderate high hydrostatic pressure (HHP) intensities at the beginning of cheese ripening has been revealed as a factor modifying proteolysis. In certain cases, HHP was able to shorten the time needed for cheese ripening. Early works suggested long treatments at moderate pressure (50 MPa for up to 72 h) as effective on the acceleration of Cheddar cheese ripening [2]. However, more recent studies showed that increments in proteolysis in Cheddar [3] and Garrotxa cheese [4] were only of small magnitude after a treatment at 50 MPa. Besides the proteolysis of Gouda cheese [5] was not significantly different after pressure treatment 50 MPa. Striking results on other cheese types remark the need of optimising the processing parameters. Hereto a short treatment at 400 MPa has been postulated as a convenient alternative to accelerate Garrotxa cheese ripening [4].

Cheese acceptability by consumers depends mainly on its organoleptic properties, striking flavour and aroma among them. Both properties are attributed to a complex combination of aromatic, volatile and non-volatile compounds [6]. The validation of any cheese ripening acceleration method should include an adequate sensory analysis. However, some methods give complementary unbiased information about the volatile spectra in cheese [7]. Simultaneous distillation and solvent extraction (SDE) is an efficient, rapid and cost-effective method to isolate volatiles from cheese. This technique is reliable and sufficiently precise, but losses of highly volatile substances may occur and the thermally induced breakdown of non-volatile precursor substances could lead to an overestimation of flavour compounds [8].

The treatment at 400 MPa at the beginning of cheese ripening has been revealed as the more promising pressure treatment for the acceleration of ripening of Garrotxa cheese [4]. This treatment produced a faster proteolysis when compared with untreated cheese. However, a deeper insight into the profile of proteolysis products showed qualitative differences with control cheeses [9]. Ripening of cheese is determined by proteolysis, but also lipolysis and glycolysis are important for texture and end flavour. This work was devoted to the analysis of the effects of high pressure treatment on the characteristic volatile profile of Garrotxa cheese after SDE and GC-MS, gaining information about proteolytic and lipolytic compounds important for cheese organoleptic properties.

Materials and methods

Cheese making and high pressure treatment

Two batches of goats' milk cheese were manufactured. Cheeses of about 1.4 kg were produced, salted in brine, and half of them were treated at 400 MPa for 5 min at 14 °C at Espuña facilities in Olot (Spain), acting the other half as control. Cheeses were ripened at 14 °C and 83% RH until sampled (21 and 60 days). A portion of cheese from each batch, treatment and ripening time (2 x 2 x 2 levels) was analysed at the Federal Research Centre for Nutrition (Karlsruhe, Germany).

Chemical composition and microbiology

Cheese was analysed for pH on a slurry 1:3 in water with a glass electrode, moisture by oven-drying at 104 °C until constant weight [10], fat by van Gulik method [11], nitrogen content by a semi-micro Kjeldahl method [12], non-casein nitrogen was measured on the fraction soluble at pH 4.4 [13], while non-protein nitrogen was measured on the fraction soluble in 12 % TCA [13].

Microbiological analysis were performed as described in Buffa et al. [14]. Starter bacteria were enumerated on M17_a (Biokar diagnostics, France) supplemented with lactose.

Volatile extraction

The simultaneous-distillation extraction (SDE) was carried out during 2 hours with 40 mL of dichloromethane gradient grade (Merck, Germany). Two hundred mL distilled water were added to 100 g goat's cheese. Differences due to extraction conditions were corrected using 5 mL of 0.33 mM citral (Merck, Germany) as internal standard. Citral is a racemic mixture of the cis-trans forms, because of that, the standard was resolved in two peaks under the chromatographic conditions performed in this study.

Remaining water was extracted with magnesium sulphide (Merck, Germany). Distillation of dichloromethane to an end volume of 1.5 mL was achieved under vacuum (210 mbar) in a rotavapor R-3000 (Buchi, Switzerland), with a vacuum controller PVK 610 (MLT, Germany)

GC-MS

One µL of the volatile concentrate was injected in a HP6890 Series GC System HP5973 fitted with a HD7683 Series Injector with split ratio 1:30 (Hewlett Packard, USA). The compounds were separated by means of a HP5MS, crosslinked 5% PHME Siloxane, 30 m length, 0.25 mm i.d., 0.25 µm film thickness column (Hewlett Packard, USA). Carrier gas flow rate was 1 mL·min⁻¹. The column oven temperature was programmed to increase from 50 °C to 280 °C at a heating rate of 4 °C min⁻¹ after 3 min at 50 °C. The eluting peaks were quantified and identified using a HP5973 Mass Selective Detector (Hewlett Packard, USA). Two separate extractions and chromatography analysis were performed for each cheese sample.

Reported results for detected compounds correspond to MS response area, corrected by cheese dry matter, and by internal standard.

Statistical analysis

Results were processed by analysis of variance (ANOVA) using the Statistica v5.0 (StatSoft Inc., Tulsa, OK, USA). The least square difference (LSD) test was used for comparison of sample data. Evaluations were based on a significance level of $p < 0.05$, unless other is stated.

Results and discussion

Overall composition

Cheese had 57.3% fat and 34.9% protein, both on dry matter basis. Moisture decreased during ripening from 49% on 3-days cheese to 33% on control cheese and to 38% on HHP-treated cheese at the end of the 8 weeks ripening time. The effect of pressure on moisture retention is produced by means of changes in internal distribution of water [15]

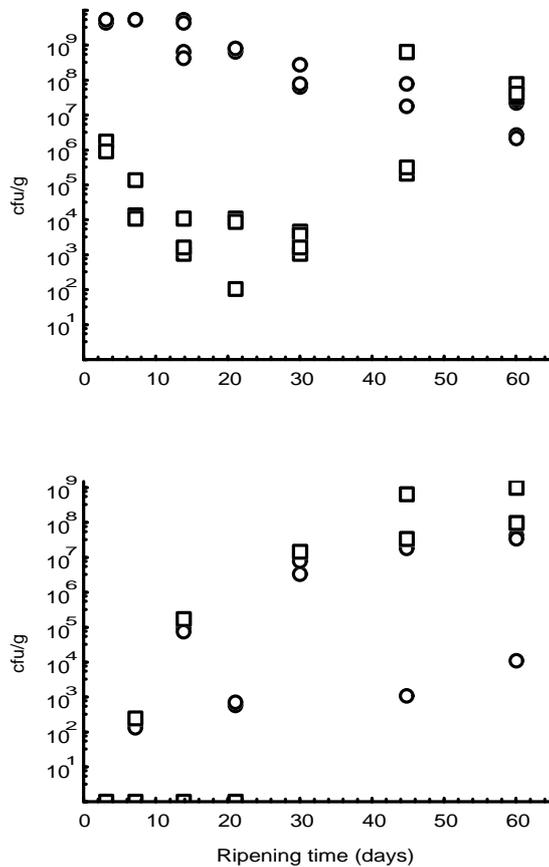


Figure 1. Microbial counts of Garrotxa cheese affected by pressure treatment and its evolution during ripening time. Top graph corresponds to starter counts (growth in M17_a), while bottom graph corresponds to NSLAB counts (growth in LBS_a). Round figures correspond to control cheese and squares correspond to HHP-treated cheese.

Together with the higher moisture content, HHP-treated cheese had higher pH ($p < 0.00001$) than control cheese. The observed difference could be due to the lack of further acidification on treated cheese after pressure treatment caused by the decrease in starter counts. The starter used was composed by *Lactococcus lactis* ssp *cremoris*, *L. lactis* ssp *lactis* y *L. lactis* ssp *lactis* biovar *diacetylactis*. Pressure treatment caused a decrease of 3 log units in starter counts, which were only slowly recovered during the first six weeks (Figure 1). This finding is in concordance with results reported on Cheddar cheese, where treatments of 400 MPa and above dramatically reduced the viability of *Lactococcus lactis* [16].

Non-casein nitrogen and non-protein nitrogen content corrected by dry matter were higher on HHP-treated cheese (Figure 2), indicating possibly an accelerated proteolysis. This effect might have been caused indirectly by pressure, since the increased pH and moisture content provide more favourable conditions for hydrolysis.

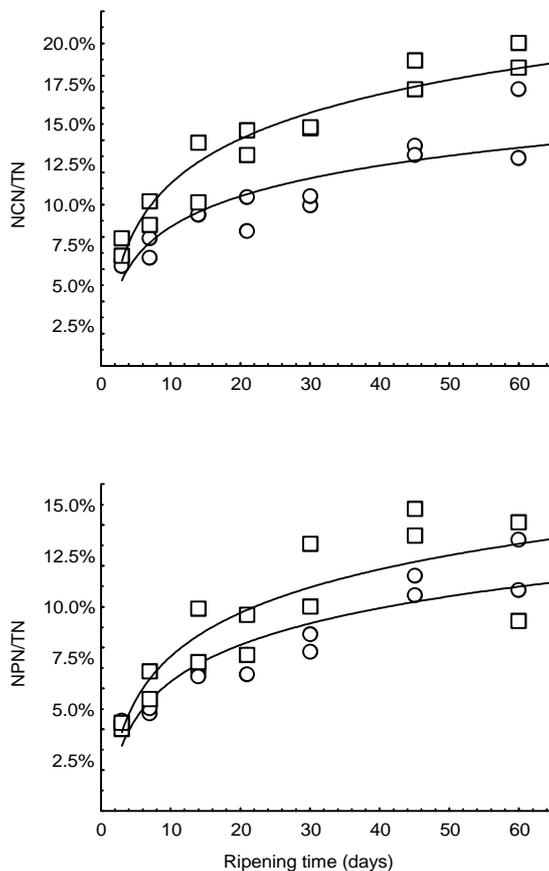


Figure 2. Evolution of proteolytic indexes during ripening of Garrotxa cheese. Top graph corresponds to Non-Casein-Nitrogen (soluble at pH 4.4) and the bottom graph corresponds to Non-Protein-Nitrogen (soluble in 12% TCA). Round figures correspond to control cheese and squares correspond to HHP-treated cheese.

Volatiles

Areas reported here are signals (mV x min) registered by the detector and they are corrected by dry matter. This correction is necessary to discriminate between a mere concentration during ripening and a real volatile production.

Twenty five different compounds were identified by MS in this study (10 fatty acids, 5 methyl ketones, 4 lactones, 3 ethyl esters, 2 aldehydes and 1 alcohol). The detected

compounds were mainly the same found by Dirinck and de Winne [17] using a similar method of analysis (50 g of cheese in 600 ml water, extracted in 50 ml dichloromethane for 4 hours) since compounds with a higher solubility in the organic solvent used for SDE will be more likely found in the extract. However, relative volatile concentrations were different as it could be expected for a different cheese type.

Free fatty acids

Fatty acids are essential elements in flavour and aroma of cheeses. Differences in fatty acids ratios are responsible for the characteristic flavour and aroma of cheese varieties [6]. Milk origin seems to have a greater importance on lipolysis products than the specificity of cheese enzymes and microorganisms. For example, the relative concentration of free fatty acids in cheese appears to be similar to those in the fatty fraction of cow's milk for Cheddar cheese [18] and goat's milk for Sainte-Maure goat's cheese [19].

Table 1. Volatile compounds in control and HHP-treated Garrotxa cheese at 21 and 60 days of ripening. Values with the same superscript letter in each row are not different by two way ANOVA ($p < 0.05$). Data in thousands of MS units, corrected by dry matter content.

		N	Ctrl 21	Ctrl 60	HHP 21	HHP 60
Even Paired Fatty Acids	Butanoic acid	16	2505 ^b	2142 ^b	1453 ^{a,b}	428 ^a
	Hexanoic acid	16	18138 ^c	15151 ^{b,c}	10215 ^b	6554 ^a
	Octanoic acid	16	18943 ^b	17686 ^b	11395 ^a	9926 ^a
	Decanoic acid	16	27160 ^a	18785 ^a	17260 ^a	14103 ^a
	Dodecanoic acid	16	4536 ^a	2373 ^a	2233 ^a	2426 ^a
	Tetradecanoic acid	11	781 ^a	318 ^a	274 ^a	613 ^a
Methyl Ketones	Heptan-2-one	15	1686 ^a	1875 ^a	1815 ^a	2297 ^a
	Nonan-2-one	16	2307 ^a	2207 ^a	2243 ^a	3055 ^a
	Undecan-2-one	16	598 ^a	594 ^a	665 ^a	721 ^a
	Tridecan-2-one	16	930 ^a	857 ^a	858 ^a	905 ^a
	Pentadecan-2-one	16	873 ^a	752 ^a	820 ^a	751 ^a
Lactones	γ -Dodecalactone	16	535 ^a	377 ^a	350 ^a	345 ^a
	δ -Dodecalactone	16	348 ^b	261 ^b	231 ^a	276 ^b
	α -Tetradecalactone	16	108 ^a	99 ^a	70 ^a	69 ^a
Aldehydes	Tetradecanal	16	88 ^a	70 ^a	75 ^a	75 ^a
	Hexadecanal	16	1379 ^a	1132 ^a	1084 ^a	1154 ^a

Milk indigenous lipase is inactivated by pasteurisation, and additionally, lactic acid bacteria used as starter in dairy products are in general only weakly lipolytic, as confirmed for Garrotxa cheese, where lipolysis is not intense [20]. Those authors measured about 6 mg of free fatty acids per gram of fat in Garrotxa cheese. On Cheddar-like hard goat cheese, increase on free fatty acids occurred mainly during the first weeks, with no significant variations after 6 weeks [21]. Also Le Quéré and colleagues [19] have found that the fatty acid profile of a soft goat cheese remained after 31 days ripening, which is in agreement with results presented on Table 1, since free fatty acids were not significantly different over the studied period, except butanoic acid. However, significant differences have been recorded when comparing the effects of treatment (Table 1). The amount of C_{4:0}, C_{6:0}, and C_{8:0} was significantly lower in HHP-cheese ($p < 0.05$). The observed reduction of 3 log units on lactococci counts on cheese treated at 400 MPa (Figure 2) as compared with fresh made cheese, might have exerted a certain influence on the concentration of fatty acids. Furthermore, pressure might have inactivated some of the lipases from secondary microbiota in cheese, which have higher activity than the starter (the most important are moulds, for their high lipolytic activity, and psychrotrophic bacteria, for their heat-stable lipases).

Table 2. Volatile compounds that are on the detection threshold in control and HHP-treated Garrotxa cheese at 21 and 60 days of ripening. Some of these compounds were detected only in few samples (no significance test could be performed on these compounds) Data in thousands of MS units, corrected by dry matter content.

	N	Ctrl 21	Ctrl 60	HHP 21	HHP 60
Heptanoic	11	144	189	185	154
Nonanoic	3	187	-	-	72
δ-Decalactone	4	27	-	55	-
2-methyl butanoic	4	-	147	-	-
3-methyl butanoic	8	88	131	79	-
Heptanol	6	31	70	46	31
Ethyl octanoate	6	63	67	176	185
Ethyl decanoate	7	98	156	316	233
Ethyl dodecanoate	2	-	-	46	-

In addition to the even-paired fatty acids reported in Table 1 some other free fatty acids have been detected. Heptanoic acid was found on 11 chromatograms, but not in HHP-treated cheese of 21 d. Nonanoic acid was detected in only 3 chromatograms, in the very detection threshold of the method.

Since short-chain fatty acids are generally found in the α position of triglycerides, the specificity of lipases can influence on their relative concentration [18]. Lactococcal lipases have preference for the α -position of triglyceride, which is the position where short-chain fatty acids are predominantly esterified [20]. The high-pressure treatment diminished bacteria counts as already reported [4] or inactivated lipolytic enzymes, reducing the amount of short-chain fatty acids released. That could have implications in

the flavour of goat's cheese, as those short-chain fatty acids have the greatest flavour impact among fatty acids, together with the branched chain fatty acids. Their flavour has been described as stale and cheesy (butanoic acid), pungent, goaty and blue cheesy (hexanoic acid), waxy, soapy, goaty, mouldy, stale and fruity (octanoic acid) [22]. High pressure could have produced a deficiency on the flavour due to the reduced production of hexanoic, octanoic and nonanoic acids, all of them having goaty flavour. However, a limited content of short and medium chain fatty acids might have a positive effect on the organoleptic characteristics of goat cheese [23], being butanoic acid responsible for a marked pungent flavour which is not always palatable to consumers. On control cheese, the detected amount of fatty acids was higher than in HHP-treated cheese, which is considered to having a mild character. In a sensory study carried out on HHP-treated Garrotxa cheese the full ripened cheese treated at 400 MPa for 5 min, as in the present study, were not scored as different in flavour intensity to the control cheese [24].

Two branched fatty acids have been detected, 3-methyl butanoic and 4-methyl hexanoic acids, both only in control samples (Table 2). 3-methyl butanoic has been described as having a cheesy flavour, playing a not negligible role on the flavour of goat cheese. These branched fatty acids are produced by the microbial activity over amino acids [25]. None of the branched fatty acids with 8 carbon atoms described as having a goat-cheese characteristic flavour has been detected after extraction with dichloromethane.

Methyl ketones

Methyl-ketones are produced from free fatty acids, by an alternative pathway to the β -oxidation. Free fatty acids are oxidised to the correspondent β -ketoacyl-CoA. The activity of a thiolase releases the ketoacid that is quickly decarboxylated by a β -ketoacyl-decarboxylase giving a methyl-ketone with one less carbon-atom than the initial fatty acid. This alternative pathway is a detoxifying way for microorganisms, removing from the environment free fatty acids by the participation of only one molecule of coenzyme A instead of the total degradation, which needs two molecules [25]. In studies where quantitative analysis were done, the ketone amount differs markedly between reports. It has been proposed a formation by thermal degradation of lipid peroxides on some SDE [8].

In Mahon cheese, the methyl-ketones of seven and nine carbon atoms were most abundant than any other methyl-ketone [26]. Table 1 shows the higher areas for the same methyl-ketones, pointing to a similar behaviour in extraction and detector response for all the compounds of this family.

Only decanoic acid content could be correlated ($p = 0.08$) with nonan-2-one. Other fatty acids have not correlated with the presence of their respective methyl-ketone. Thus methyl-ketone production seems to be not limited by the substrate concentration.

Two methyl-ketones have been described as having dairy flavours (heptan-2-one has a blue cheese flavour and tridecan-2-one has heated milk-like flavour [27]). Nonan-2-one has a flavour described as fruity and floral while undecan-2-one has been associated to citrus or rose flavours [27]. The effect of treatment was not noticeable on methyl ketones amount, except for the longest chains. Tridecan- and pentadecan-2-one were found in lower concentration in HHP-treated cheese ($p = 0.01$ and $p = 0.09$ respectively). Formation of methyl-ketones is mainly a result of the lipolytic action of the microbiota in the cheese [28]. The microbial counts were reduced by the effect of HHP treatment. The starter counts were reduced by 3.4 log units right after the

treatment. A reduction of 3.4 log cycles in 400 MPa treated Cheddar cheese has been reported [16]. In spite of the latter recovery, at day 21 the difference was still of 2 log units. At day 60 the reduction on starter counts in control cheese, together with the recovery on the counts in HHP-treated cheese, make the difference disappear. The recovery on counts did not ensure the recovery on the composition of cheese microbiota, but it can be presumed a decrease on the microbial production of volatiles, at least on the first weeks.

All the ketones detected increased their concentration within the two ripening stages studied (Table 1). Mulet et al. [26] also found an increase in heptan- and nonan-2-one during ripening of Mahon cheese, being the concentration of undecan-2-one not significantly affected by ripening time.

Lactones

Lactones are produced from hydroxylated fatty acids. They are cyclic under acidic conditions, or by the activity of microorganisms. Cycles have 5- or 6-carbon atoms, giving γ - and δ -lactones respectively. Lactones are known to contribute to a buttery character in cheese [17].

δ -Tetralactone, γ -, and δ -dodecalactone were found in all the samples, being their concentration not affected by the treatment applied (Table 1). Their amount increased significantly between day 21 and 60. δ -Decalactone was found only in samples of 21 d (Table 2).

Detection threshold of δ -Lactones is usually than γ -lactones. The activity of microorganisms on lactone production in cheese has never been clearly explained [25].

Esters

The reaction of free fatty acids with alcohols yields esters with fruity flavours. Free fatty acids are product of lipolysis and alcohols, like ethanol, are products of lactose metabolism. A large range of enzymes are involved on esterification reactions, like alcohol-acyltransferases.

Three ethyl esters were detected in Garrotxa cheese during this study: Ethyl octanoate and ethyl decanoate were present only in 21-d samples, and the concentration was not different between the two treatment-groups (Table 2). However, ethyl dodecanoate was detected only in some 60 HHP-treated samples.

Aldehydes

Products of the β -oxidation of unsaturated fatty acids, usually associated with green-grass-like and herbaceous aromas [29].

Two long chain aldehydes were found in the extract. Tetradecanal and hexadecanal concentrations were significantly higher in the oldest samples (Table 1). No differences due to treatment were recorded on aldehyde concentrations.

Alcohols

Alcohols are difficult to detect using SDE as extraction method in cheese samples. The only alcohol detected was heptan-2-ol, as in Mahon cheese [26]. It was present only in 60 d samples and its concentration was significantly lower in HHP-treated cheese (Table

2). Alcohols are products of the reduction of methyl ketones by the action of reductases. Many other reactions in cheese can yield alcohols as a final product, like amino acid metabolism by Ehrlich pathway, an aldehyde reduction among others [25]. Heptan-2-ol is usually present in important amounts together with heptan-2-one, the correspondent methyl ketone.

Conclusions

Twenty five components of the volatile fraction of Garrotxa cheese have been extracted with a simultaneous distillation-extraction method and further analysed and identified by GC-MS. HHP-treatment of a mild goat's cheese has produced negligible changes on the volatile composition extractable with dichloromethane, up to the range assayed during this work. No new compounds have been detected on HHP-treated cheese. The only changes observed on the volatile fraction are on the response area recorded for the extracted compounds. Even this difference is scarce on cheeses after 60 days of ripening. From this point of view substantial equivalence might be achieved and pressure can be regarded as a safe technology.

Lipolysis releases fatty acids associated with a typical goat's flavour (4-methyl-octanoic, 4-ethyl-octanoic, hexanoic, octanoic, nonanoic and decanoic), but an excessive lipolysis has been observed as a cause of rancid flavours [30]. Lipolysis was less intense in high pressure treated cheeses.

Proteolysis has been usually regarded as the main process involved in cheese ripening. A previous work has shown that the treatment at 400 MPa is able to produce a higher amount on proteolytic products by the end of ripening [9]. Results were confirmed here for non-casein nitrogen and non-protein nitrogen.

However, a sensory test carried out on cheeses equivalent to those of the present study, no significant differences have been recorded between controls and 400 MPa-treated cheeses [4].

Acknowledgements

Authors acknowledge technical assistance given by W.D. Koller and U. Wagner from the Institut für Verfahrenstechnik, Karlsruhe (Institute of Process Engineering).

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