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Effects of High Hydrostatic Pressure Treatments on Biogenic Amine Contents in Goat Cheeses during Ripening

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The application of high-pressure treatment to accelerate cheese ripening may influence the occurrence and profile of biogenic amines. In some cases, high-pressure treatment could yield high amine content, whereas in others the amine concentrations were comparable with those in nontreated ones. Tyramine was the most affected amine. However, the influence of high pressure depended on the treatment applied. The highest amine concentrations were observed when 50 MPa for 72 h was applied, and in which, the content of tyramine was almost three times higher than in the untreated samples. On the contrary, when 400 MPa for 5 min or 400 MPa for 5 min plus 50 MPa for 72 h were applied contents of this amine were similar or lower than those found in control cheeses showing similar degrees of proteolysis. The higher proteolysis, induced by the pressurization treatments, was not correlated with the higher amine production.

KEYWORDS: Biogenic amines; polyamines; cheese; high-pressure; ripening; tyramine; spermidine; cadaverine; putrescine; histamine

INTRODUCTION

Cheese ripening is a complex biochemical process involving proteolysis, lipolysis, and glycolysis. For most cheese varieties, proteolysis is the most important reaction during cheese ripening, being responsible for both textural changes and flavor development (*1*). The large facilities required to stock the cheese during the ripening period, the cost of refrigeration, as well as the weight loss and the associated capital immobilization make the ripening a slow and expensive process. High-pressure-based methods have been proposed by several authors to accelerate cheese ripening (*2*, *3*). The addition of exogenous enzymes or cheese slurries, and the use of proper starters or adjunct cultures have also been reported to shorten cheese-ripening times. However, some of these procedures have some drawbacks. Thus, the distribution of enzymes on cheese curd and the control of their action during ripening is difficult. This problem can be partially solved by enzyme encapsulation, but the effectiveness of the process is still low and the cost is high. In addition, in some countries, there are legal barriers to the use of some of these procedures for cheese ripening (*2*). High-pressure treatment can be an alternative to those methods of ripening acceleration or it can even be used in combination with them.

High-pressure treatment changes the cell membrane permeability of starter bacteria and thus endocellular material, such as peptidases, are released to the medium. On the other hand, it also causes some changes in the cheese matrix, such as an increase in water retention, and pH, that can contribute to proteolysis, thus reducing the ripening time.

Since 1992, Yokohama et al. (*4*) have had patent about cheese-ripening acceleration by using a high-pressure treatment. These authors claimed to obtain a product comparable with a 6-month-old commercial cheese by applying a pressure treatment to Cheddar cheese at 50 MPa and 25 °C for 72 h. However, the level of adjunct lactobacilli added was not comparable with that of the standard Cheddar cheese manufacture protocol, and the applied production temperature was also different: 25 °C in pressure-treated and 7.2−11 °C in conventional ripening for Cheddar. Results reported for application of 50 MPa for 72 h are very different. Thus, this treatment applied on conventional Cheddar only produced a very small increase in ripening (*5*). However, the same pressure conditions yielded an important increase in proteolysis in Pere Joseps Cheese (*6*). For Camembert cheese, Kolakowski et al. (*3*) found a significant increase of pressure on proteolysis, having a maximum effect for 50-MPa treatment during 4 h, but they found only a slight increase in proteolysis when Gouda cheese was exposed to high pressure (from 50 to 500 MPa). In goat cheese, the usefulness of accelerating ripening by a combined high-pressure procedure has been reported. This procedure involves an initial shock treatment at 400 MPa for 5 min to release microbial enzymes, followed by a 50-MPa treatment for 72 h, to increase the activity of previously released enzymes (*2*).

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High-pressure treatment of cheese affects the proteolysis during ripening involving an increased production of small peptides and free amino acids (*7*). The increase in free amino acids could favor the production of biogenic amines, because some amino acids, such as tyrosine and histidine, are precursors of biogenic amines, such as tyramine and histamine. Biogenic amines are aliphatic, aromatic, or heterocyclic organic bases of low molecular weight, which are formed as a consequence of microbial amino acid decarboxylation reactions (*8*). Due to their significance in the fermentation process, lactic acid bacteria have been reported as being capable of biogenic amine formation in fermented foods (*9*). Biogenic amines are classified as: aromatic biogenic amines (octopamine, dopamine, tyramine, serotonin, histamine, β-phenylethylamine, and tryptamine); diamines (putrescine and cadaverine); and polyamines (agmatine, spermidine, and spermine).

Small amounts of biogenic amines can be found in a variety of food and are considered safe for consumption. However, the intake of large quantities of some amines can lead to toxicological effects, such as headache, nausea, respiratory distress, heart palpitations, hyper- or hypotension, and anaphylactic shock syndrome (*10*, *11*). Moreover, these effects can be especially adverse in patients treated with classical monoamine oxidase inhibitors drugs (MAOIs) (*12*) or in individuals with genetic or acquired diaminoxidase deficiency.

Data about the effect of high hydrostatic pressure on biogenic amine contents in food are very scarce, and no data have been found for cheeses, in particular. In seafood high pressure from 150 to 400 MPa applied to extend the shelf life yields an increase or a decrease depending on the biogenic amine considered (*13*).

The purpose of this study was to monitor the effects on biogenic amines of a treatment combining microbial enzyme release, as a consequence of the application of a high-pressure/short-time treatment (400 MPa/5 min), and the increase in enzyme activity, as a result of a long-time/moderate high-pressure treatment (50 MPa/72 h), applied to accelerate cheese ripening. Likewise, the particular effects of both treatments when they were applied separately were also studied to better understand the way of each high-pressure treatment could affect the biogenic amine contents in cheese. The high-pressure acceleration of cheese ripening would not be advisable if the level of biogenic amines increased dramatically, due to their potential toxic effects. In addition, the influence of high-pressure treatments on the proteolysis indexes and lactic acid bacteria were also studied to evaluate their relationship with biogenic amines.

MATERIALS AND METHODS

Cheese Manufacture. Cheese was manufactured from goat milk in the CeRTA-Food Technology Pilot Plant at the Autonomous University of Barcelona. Pasteurized (72 °C, 15 s) milk was inoculated with (2% vol/vol) homofermentative starter (*Lactococcus lactis* ssp *cremoris*, *Lactococcus lactis* ssp *lactis*, *Lactococcus lactis* ssp *biovar diacetylactis*, AM Larbus S. A., Barcelona, Spain). Calf rennet (0.07 mL/L, containing 780 mg chymosin/L) and calcium chloride (0.056 g/L) were used as coagulating agents. Coagulation occurred at 30 ± 1 °C within approximately 45 min, and then curd was gently cut into 8- to 10-mm cubes. The curd was held for 5 min before stirring and further warming to 32 °C. When curds and whey reached the desired temperature they were held for 15 min, then the whey was immediately drained from the vat. Drained curds were molded into cylindrical holders and pressed in a pneumatic press at 0.27 MPa for 30 min and then 0.55 MPa for 4 h. The molds were 5 cm deep by 9 cm in diameter and yielded pressed curds weighing about 250 g each. Cheeses were salted in brine (16% sodium chloride, 14 °C) for 45 min. Regular ripening conditions are 14 ± 1 °C and 86 ± 2% relative humidity. High-pressure treatment was done on day 1 after cheese making.

Samples. Cheese productions were divided into batches to be treated under different high-pressure conditions: untreated cheese as a control; treatment 1, 400 MPa for 5 min; treatment 2, 50 MPa for 72 h; treatment 3, 400 MPa for 5 min and 50 MPa for 72 h. The treatments were done at 14 °C (the same temperature as the ripening chamber). A total of 45 samples belonging to six different cheese productions were studied. The three first productions included two batches (control and treatment 2) and the other three productions included three batches (control, treatment 1, and treatment 3). Every batch consists of three cheeses, which were sampled in duplicate at 4, 14, and 28 days of ripening. The determination of free amino acids was only performed in cheeses from control and from treatments 1 and 2, and microbial counts were only recorded in control samples and in those of treatment 1. All pieces were vacuum packaged individually in a low-water and oxygen-permeability barrier film (Cryovac BB4L, Grace, Italy) to avoid contact between the cheese and the pressure transmission medium during the pressurization treatment. After 72 h packing material was removed from all cheeses.

High-Pressure Processing. Pressurization was performed by using discontinuous high hydrostatic pressure equipment (Alstom, Nantes, France), with a pressure chamber of 2 L capable of reaching 500 MPa in 2 min. Cheeses were immersed in water, which acted as a hydrostatic fluid medium, and pressurized according to the conditions described above. The chamber temperature was adjusted by means of a thermoregulating system involving the circulation of a cooling fluid (ethanol−water mixture) within the walls of the vessel. The selected temperature of 14 °C was checked in the water inside the vessel by using a thermocouple. Samples were placed in the chamber for 5−10 min at atmospheric pressure until temperature equilibrium was established. The pressure-releasing speed was manually adjusted to always spend the same time to achieve atmospheric pressure: approximately 100 s for 400 MPa and 10−15 s for 50 MPa.

Determination of Biogenic Amines. The biogenic amines were separated and quantified by the HPLC method by Novella-Rodríguez et al. (*14*). The method consists of the separation of the ion pair formed between biogenic amines and octane sulfonic acid on a reverse-phase column, using postcolumn derivatization with *o*-phthalaldehyde and spectrofluorometric detection. The biogenic amines were extracted from cheese samples with 0.6 N perchloric acid. All reagents were analytical grade except the HPLC reagents that were liquid chromatographic grade. Biogenic amine standards were purchased from Sigma Chemical (St. Louis, MO). Analyses were performed in duplicate, and the results of biogenic amines were expressed in dry weight.

Chemical Analysis. Moisture and total nitrogen were calculated on the basis of International Dairy Federation−FIL standard procedures (*15*, *16*). The nonprotein nitrogen, soluble in 12% trichloroacetic acid, was performed according to McSweeney and Fox (*17*). The proteolysis index was calculated as the quotient between nonprotein nitrogen and total nitrogen multiplied by 100. The concentration of individual free amino acids was determinated in the 2% trichloroacetic acid-soluble fraction by reverse-phase-HPLC (*18*).

Bacteriological Analysis. Lactococci were enumerated on M17 agar plates (Biokar Diagnostics, Beuvais, France), supplemented with lactose (Carlo Erba, Milano, Italy), and lactobacilli on Rogosa agar (Biokar) both incubated at 30 °C in microaerophilia.

Statistical Treatment. Friedman's test and Wilcoxon's test for nonparametric data were used to evaluate the contents of biogenic amine in cheese, because data did not follow the normal distribution. All statistical analysis was performed using the Statistical Software Package for Windows 9.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Figure 1 shows the average of the proteolysis index and their corresponding deviations in control and high-pressure-treated samples after 4, 14, and 28 days of ripening. Proteolysis indexes were after 28 days of ripening higher in pressurized samples than in control ones. Proteolysis values recorded suggested that,

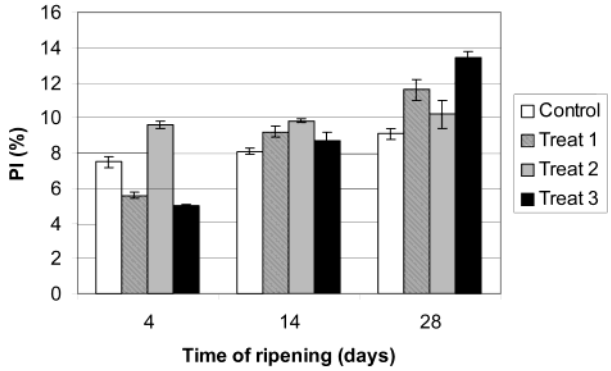

Figure 1. Profile of proteolysis indexes throughout ripening in cheese with and without high-pressure treatment. Treatment 1, 400 MPa for 5 min; treatment 2, 50 MPa for 72 h; and treatment 3, 400 MPa for 5 min followed by 50 MPa for 72 h.

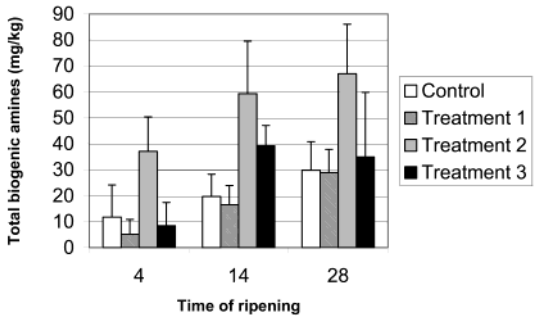

Figure 2. Profile of total biogenic amine contents throughout ripening in cheese with and without high-pressure treatment. Treatment 1, 400 MPa for 5 min; treatment 2, 50 MPa for 72 h; and treatment 3, 400 MPa for 5 min followed by 50 MPa for 72 h.

as it was previously reported by other authors (*2*), the high-pressure treatments assayed favored the ripening, being the values of treated samples at day 14 similar to those of the control at day 28.

Figure 2 shows the values of total biogenic amines that increased throughout ripening. Because results are expressed on a dry-weight basis, the increase is due to a real production and not for concentration effect. This agrees with studies from various authors (*19*, *20*) reporting the accumulation of some biogenic amines during conventional cheese ripening. However, the formation of biogenic amines was different according to the treatment applied at the beginning of ripening. Thus, cheeses obtained from the same pasteurized milk but processed with high-pressure treatments differ in their biogenic amine concentration. The biogenic amine values recorded in the control batch (without high-pressure treatment), were lower than those found in cheeses with high-pressure treatments 2 and 3, whereas biogenic amines from treatment 1 were similar or lower than in the control samples. These differences were also found when comparing biogenic contents of control cheese at day 28 and high-pressure-treated cheeses having a similar proteolysis index (day 14). Therefore, both high-pressure treatments 2 and 3 yielded an increase in the biogenic amine concentration. The highest amine content was found in cheeses from treatment 2.

To confirm the findings above, Friedman's test was applied for nonparametric data, and statistical differences ($P < 0.05$) were found in the total content of biogenic amines among cheeses from the control group and the high-pressure treatments 2 and 3. To evaluate the differences between control and each one of these two high-pressure treatments, Wilcoxon's tests were

Table 1. Aromatic Amines, Diamines, and Polyamines Contents (mg/kg dw) in Cheese with and without High-Pressure Treatment

amines	control	400 MPa	50 MPa	400 + 50 MPa
tyramine	10.3 (5.8)[a]	1.1 (1.7)	28.5 (3.1)	1.6 (2.5)
histamine	1.4 (1.2)	1.0 (0.9)	4.0 (1.0)	1.5 (1.4)
total aromatic amines[b]	13.4	2.1	35.5	6.2
putrescine	0.3 (0.3)	nd[c]	6.8 (5.4)	4.2 (0.6)
cadaverine	0.4 (0.2)	nd	2.8 (0.7)	1.7 (0.6)
total diamines[b]	0.7	nd	9.6	5.9
spermidine	14.7 (5.7)	26.4 (7.9)	17.9 (9.2)	20.2 (13.9)
spermine	0.9 (0.3)	1.0 (0.1)	3.5 (0.2)	2.5 (2.6)
total polyamines[b]	15.7	27.4	21.9	22.8

Period of ripening: 28 days. [a] Mean (standard deviation). [b] Sum of all amines. [c] nd = not detected.

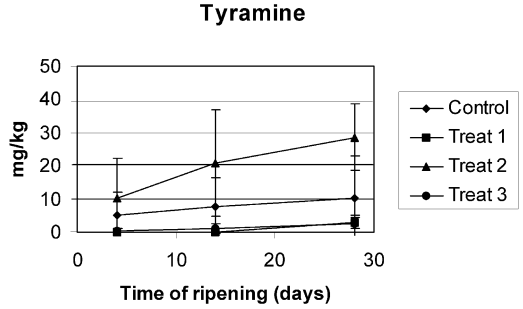

Figure 3. Profile of tyramine throughout ripening in cheese with and without high-pressure treatment. Treatment 1, 400 MPa for 5 min; treatment 2, 50 MPa for 72 h; and treatment 3, 400 MPa for 5 min followed by 50 MPa for 72 h.

applied and statistical differences ($P = 0.002$) were found only between control and treatment 2.

Despite the variability, the main amine found was tyramine, especially in treatment 2 (**Table 1**). In order of concentration, the other aromatic amines detected were histamine, tryptamine, and β-phenylethylamine. The concentrations of tryptamine and β-phenylethylamine were less than <1 mg/kg dry weight (dw). The remaining amines, such as octopamine and dopamine, were negligible or at low concentrations (<0.5 mg/kg dw). Moreover, serotonin was not found in any tested samples. In contrast to other works, where diamines were the major amines in ripened cheeses (*19*, *20*), putrescine and cadaverine were found at low contents (**Table 1**) and in few samples. Putrescine was only detected in 25% of the total samples studied, whereas cadaverine was detected in 12% of them. However, as for tyramine, the highest concentrations were also found in treatment 2 samples. Concerning polyamines, spermidine was clearly the most important polyamine, followed by spermine, whereas agmatine was only found in a few samples at low concentrations <1 mg/kg dw.

The behavior of the main amine, tyramine, was dependent on the high-pressure treatment applied. As **Figure 3** shows, tyramine presented a high variability among treatments, so treatment 2 (50 MPa) lets tyramine increase, whereas treatments 1 and 3, in which a pressure of 400 MPa was applied, tyramine contents were lower than in control cheeses. As also happened for the total contents of biogenic amines differences among control and pressurized cheeses were also observed when comparing high-pressure-treated samples with controls having a similar degree of proteolysis. The influence of high-pressure treatment was less evident for the other aromatic amines, because they consistently remained at low concentrations. Spermidine, which was the major polyamine found in cheese samples from 1 day of ripening, remained as the more significant

Table 2. Composition of Free Amino Acids (mg FAA/100 g dw) in Cheese with and without High Pressure

	control			400 MPa			50 MPa		
	4 days	14 days	28 days	4 days	14 days	28 days	4 days	14 days	28 days
FAA[a]	242.8 (27.9)[b]	229.7 (63.0)	323.1 (60.2)	168.1 (58.9)	344.2 (74.5)	644.8 (93.8)	204.1 (59.1)	313.3 (27.1)	472.1 (52.8)
TYR[c]	10.4 (1.3)	11.1 (1.8)	11.1 (2.0)	7.6 (1.9)	11.4 (3.1)	19.2 (3.5)	11.4 (3.0)	11.1 (2.4)	13.2 (2.3)
HIS[d]	9.3 (0.5)	9.5 (1.2)	11.9 (1.7)	6.5 (0.1.3)	11.3 (3.7)	13.9 (1.1)	8.8 (1.6)	14.6 (0.1)	12.8 (0.8)
PHE[e]	15.5 (0.4)	20.0 (1.8)	23.5 (1.7)	10.9 (2.7)	29.8 (3.8)	57.5 (1.4)	13.2 (1.5)	26.3 (3.1)	29.4 (0.1)
LYS[f]	11.5 (1.9)	10.3 (2.1)	16.1 (2.0)	7.7 (1.3)	12.1 (1.4)	23.5 (1.8)	8.6 (1.6)	13.4 (3.2)	16.9 (3.1)

[a] FAA = free amino acids. [b] Mean (standard deviation). [c] TYR = tyrosine. [d] HIS = histidine. [e] PHE = phenylalanine. [f] LYS = lysine.

polyamine throughout the entire ripening process, irrespective of the high-pressure treatment applied. The contents of spermidine at the end of ripening were higher in samples of treatment 1 (26.5 mg/kg dw) than in the control (14.7 mg/kg dw), but differences were not statistically significant.

The high-pressure application of 50 MPa for 72 h (treatment 2) to accelerate the cheese ripening could lead to a larger formation of biogenic amines, particularly tyramine, in comparison with control cheeses manufactured under the same conditions but without high-pressure treatment. However, the application of other high-pressure conditions (treatments 1 and 3) yielded cheeses with lower tyramine contents than the control. This could be explained by the occurrence of the different effects of high pressure on enzyme activity and microbial growth. Thus, high pressure could provoke both inactivation or activation of enzymes, depending on the treatment intensity but also on the type of enzymes, the nature of the substrates, the temperature, and the length of processing (*21*, *22*). Therefore, every enzyme seems to react differently depending on the high pressure applied (*22*). In addition, the volume reduction associated to high-pressure treatment (*21*) could lead a substrate−enzyme approach favoring the amine formation.

The higher concentrations of biogenic amines in pressurized samples could be related to an increase of proteolysis yielding a greater free amino acids liberation throughout ripening. As it was shown above (**Figure 1**), the high-pressure treatments of cheese produce changes on the degree of proteolysis, but the extent of the changes depends on the pressure treatment applied. The proteolysis indexes were higher in pressurized than in control cheeses, especially when the treatment 3 was applied. This fact agrees with the release of proteolytic microbial enzymes linked to treatment at 400 MPa and to the increase of enzyme release activity during the 72 h a pressure of 50 MPa was applied. However, the higher degree of proteolysis, expressed as proteolysis indexes, found for treatment 3 does not agree with the higher amine formation, which was observed in treatment 2.

A high presence of free amino acids has been also related to high biogenic amine content; however, this relationship was not observed here. Only the pressurized samples with treatment 1 showed a statistically significant higher level of total free amino acids, particularly at the end of the ripening (**Table 2**). This difference can be related to the fact that treatment at 400 MPa seems to increase proteolysis throughout the whole ripening, whereas treatment at 50 MPa only increases proteolysis during the time that it is applied. Moreover, treatment at 50 MPa is especially effective to liberate primary proteolysis product rather than free amino acids, whereas in the treatment at 400 MPa, the increase of proteolysis can be attributed mainly to the releasing of microbial enzymes (*2*).

Regarding biogenic amines, cheeses from treatment 1, with higher amounts of free amino acids, did not show contents of biogenic amines statistically different from those of the control.

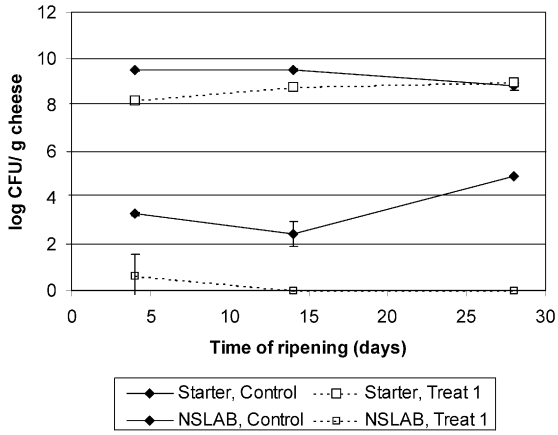

Figure 4. Profile of starter and nonstarter lactic acid bacteria in control samples and samples from treatment 1 throughout ripening. NSLAB: nonstarter lactic acid bacteria; treatment 1, 400 MPa for 5 min.

Despite the higher formation of tyramine in pressurized samples from treatment 2, the corresponding concentrations of tyrosine were similar to the control samples. In contrast, tyrosine in samples of treatment 1 was higher and this was not linked to a higher tyramine production. Likewise, phenylalanine was the main amino acid found and this cannot be correlated with a significant formation of β-phenylethylamine, which was always found at concentrations lower than 1 mg/kg. Therefore, although free amino acids are described as stimulators of the decarboxylative activity of bacteria, their levels do not seem to be a critical factor for explaining differences in biogenic amine formation.

Another factor involved in biogenic amine production is the occurrence of active amino acid decarboxylating bacteria. To check if pressurization affects these microbial counts, starter and nonstarter lactic acid bacteria counts were studied in control and in samples from treatment 1. The amino acid decarboxylase activity of lactic acid bacteria has been described, especially for tyramine (*24*−*26*). As **Figure 4** shows, the initial counts of starter were lower than in control, but at the end of the ripening, the counts were very close. However, the behavior of nonstarter lactic acid bacteria was different. In control there was an increase throughout ripening, but the counts were always low in pressurized samples. Therefore, high pressure reduced the occurrence of nonstarter lactic acid bacteria, which is in agreement with the lower tyramine content in samples from treatment 1 when compared with control samples.

Aromatic amine contents in cheeses studied here were much lower than the concentrations reported as toxic. It is assumed that 2−10 mg histamine per person and day is compatible with humans (*20*) and a portion of 200 g of cheese would contribute only with less than 0.2 mg. It is considered that 100−125 mg tyramine could cause migraine, and it is far from the 3 mg of a 200-g portion. People following a treatment with classic MAOI drugs is much more sensitive to amines, but the amine content

in analyzed cheeses is still below the 6 mg that would cause a slight blood-pressure increase.

In conclusion, the use of high pressure for ripening acceleration does not seem to affect (treatment 3) nor even reduce (treatment 1) the total biogenic amine content of cheeses when pressures of 400 MPa are applied. In contrast, the use of prolonged treatment at 50 MPa could be the less suitable. Despite the general association between higher proteolysis and higher amine formation, this relationship was not so evident in this work. The higher tyrosine concentrations did not produce the higher tyramine contents. Further studies should be conducted to confirm the influence of high-pressure treatment on the fermenting and wild microorganisms, as well as on the biogenic amines behavior during cheese ripening. However, the results of this work are promising and they could be helpful to obtain cheeses with low biogenic amine contents, which would be particularly appropriate for patients under MAOI drug therapy or for individuals having special sensibility to these compounds.

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