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Biogenic Amine Formation and Nitrite Reactions in Meat Batter As Affected by High-Pressure Processing and Chilled Storage

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Changes in biogenic amine formation and nitrite depletion in meat batters as affected by pressure−temperature combinations (300 MPa/30 min/7, 20, and 40 °C), cooking process (70 °C/30 min), and storage (54 days/2 °C) were studied. Changes in residual nitrite concentration in raw meat batters were conditioned by the temperature and not by the pressure applied. Cooking process decreased ($P < 0.05$) the residual nitrite concentration in all samples. High-pressure processing and cooking treatment increased ($P < 0.05$) the nitrate content. Whereas protein-bound nitrite concentration decreased with pressure processing, no effect was observed with the heating process of meat batters. High-pressure processing conditions had no effect on the rate of residual nitrite loss throughout the storage. The application of high pressure decreased ($P < 0.05$) the concentration of some biogenic amines (tyramine, agmatine, and spermine). Irrespective of the high processing conditions, generally, throughout storage biogenic amine levels did not change or increased, although quantitatively this effect was not very important.

KEYWORDS: Residual nitrite; nitrate; nitrite-bound protein; biogenic amines; high pressure; meat batters

INTRODUCTION

High pressure is applied in muscle-based food processing because of its effects on food constituents and primarily on microorganisms (spoilage and pathogens) (*1*−*3*). High-pressure processing can affect the development of different chemical and enzymatic reactions because they often involve a change in volume (*4*). The application of high pressure can have detrimental effects on microbial physiology and viability, damaging and even inactivating cells, thus enhancing food safety and prolonging shelf life. In both instances, the high-pressure processing effect depends on several factors, among others, those associated with the nature of the medium, or the high-pressure processing conditions, of which temperature is particularly important. Different pressure/temperature combinations have been used in new applications in the food industry.

Biogenic amines are toxic compounds formed by decarboxylation of free amino acids (FAAs) from the action of amino acid decarboxylase enzymes. Biogenic amine concentration is conditioned by numerous factors such as FAA content and availability, microorganisms capable of producing decarboxylases, the nature of the medium (pH, ion strength, etc.), and processing and storage conditions, etc. (*5*, *6*). In fact, the induced effects of high pressure on complex biological systems can affect several of the factors conditioning biogenic amine formation (among others, FAA levels or enzymatic activity) (*6*). Changes in protein structure are associated with changes in volume and may therefore be affected by pressure, causing changes in enzymatic activity. Ohmori et al. (*7*) showed that high-pressure processing (100−500 MPa/10 min/25 °C) increased the FAA content of beef, which may be the result of an increase in endogenous proteolytic activity. Different levels of biogenic amines have been reported in pressurized meat products, including cooked meat emulsion (*8*).

Because the nitrite added to meat products interacts with several components of the complex biological systems, it is known to become rapidly depleted depending on factors such as initial nitrite concentration, presence of reductants, acidity, product composition, and processing and storage conditions (*9*, *10*). In spite of the desirable benefits of nitrite, there has been controversy over its use as a meat-curing agent, partly because of its potential to react with amines and amides to form carcinogens and partly because of its contribution as a source of nitrite in human nutrition (*11*). Recently, it has been highlighted that residual nitrite found in cured meat products has been substantially reduced (as much as 80%), thanks to, among other factors, changes in the manufacturing process (*12*, *13*). High-pressure processing conditions can affect different physicochemical characteristics of cooked meat systems. For example, thermal protein denaturation of meat batters (in the range of the usual cooking temperature) was pressure−temperature interdependent (*14*). It is therefore plausible that pressure−temperature combinations could condition nitrite reactions in these processed meats. Changes in residual nitrite in pressurized meats have been studied by some authors (*15*), although no data have been reported.

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Many of the factors that promote or inhibit biogenic amine formation and the chemical behavior of nitrite in meat products can be considerably altered by the application of high pressure (*16*). Because no data are available, more research is needed to evaluate the impact of high-pressure processing on the presence of these compounds. This knowledge would therefore be useful for the technological and biochemical aspects. The aim of this study was to evaluate how the application of different pressurization conditions (300 MPa/30 min at 7, 20, or 40 °C) affects biogenic amine formation and nitrite depletion of meat batters during heat processing (70 °C/30 min) and subsequent chilled storage (54 days/2 °C).

MATERIALS AND METHODS

Meat Raw Materials and Additives. The formulations were prepared from post-rigor pork meat (mixture of M. biceps femoris, M. semimembranosus, M. semitendinosus, M. gracilis, and M. aductor) and pork backfat obtained from a local market. Visible fat and connective tissue were trimmed from pork meat, and both lean pork and backfat were separately ground through a 6-mm plate, vacuum packed (1 kg), and frozen at −20 °C until product formulation, which took place within 3 weeks.

Additives used for the preparation of meat batters included sodium chloride (2.5%), sodium tripolyphosphate (STP) 0.18%, and 150 ppm of sodium nitrite (Panreac Química, S.A., Barcelona, Spain).

Meat Batter Preparation, High-Pressure Processing, and Chilled Storage Conditions. Meat and fat packages were thawed (for approximately 18 h at 3 ± 2 °C, reaching between −3 and −5 °C). Raw meat material was homogenized and ground for 1 min in a chilled cutter (2 °C) (Stephan Universal Machine UM5, Stephan u. Sóhne GmbH & Co., Hameln, Germany). Sodium nitrite, NaCl, and STP were dissolved in water and chilled (2 °C); this solution was added to the meat and the whole mixed again for 1 min. Finally, the fat was added and homogenized all together for 1 min, and then during 2 min more the mass was homogenized in vacuum conditions. Mixing time was standardized to 5 min, and the final temperature of the meat batter was below 10 °C.

The batter was placed in flexible plastic jars (diameter = 33 mm) containing 60 ± 0.5 g, taking special care to avoid trapping air. These jars were randomly divided into two groups: nonpressurized (control, C) and pressurized samples. For pressure treatment each jar was hermetically sealed and placed in a 8 cm × 30 cm Ultra-Cover latex bag (Amevisa S.A., Madrid, Spain). Pressure−temperature processing was carried out on a high-pressure pilot unit ACB model AGIP 665 (GEC, Alsthom, Nantes, France) using water as the pressurizing medium at 300 MPa/30 min [on the basis of the previous results of López Caballero et al. (*17*)], and three different pressure temperatures were used: 7 °C (HP7); 20 °C (HP20); and 40 °C (HP40).

After pressurizing treatments, the samples (HP7, HP20, and HP40) were removed from the latex bags, and together with the nonpressurized sample (C) were cooked at 70 °C during 30 min in a water bath. Then all of the samples were stored at 2 ± 1 °C (darkness) for 54 days and analyzed periodically. Raw and heated meat batters (nonpressurized and pressurized) were also analyzed immediately after formulation, high-pressure processing, and heating treatment.

Proximate Analysis and pH. Representative batters were analyzed for moisture and ash contents (*18*) and protein content by using a LECO FP-2000 Organic Nitrogen Determinator (Leco Corp., St. Joseph, MI) in quadruplicate. Fat content was evaluated in duplicate according to the method of Bligh and Dyer (*19*). The pH was determined in duplicate using a pH-meter (Radiometer PHM 93, Copenhagen, Denmark) on a homogenate of 10 g of sample in 100 mL of distilled water.

Determination of Residual Nitrite and Nitrate by Flow Injection Analysis (FIA). The determination of residual nitrite and nitrate contents in the cooked samples was performed using the FIA technique according to the method of Ruiz-Capillas et al. (*20*). The extract used for determinations was prepared from 10 g of the sample according to the AOAC method (*18*) with a final volume of 250 mL. This extract of samples was injected into the FIA equipment as was described in Ruiz-Capillas et al. (*20*). Nitrate was reduced to nitrite using a cadmium redactor (FOSS Tecator, Sweden) placed in the FIA system. Nitrate content was determined by differences between the nitrite content after the reducing process and the residual nitrite. Standard nitrite and nitrate solutions with concentrations from 0.125 to 4 mg of NO_2 and NO_3/L were prepared from a stock solution of 1000 mg of NO_2 and NO_3/L. Results are averages of at least three determinations.

Determination of Protein-Bound Nitrite (PBN) by FIA. PBN determination was based on the method of Olsman and van Leeuwen (*21*) as described by Ruiz-Capillas et al. (*20*). The results (means of at least three determinations) were expressed as milligrams of NO_2 per kilogram of sample.

Analysis of Biogenic Amines by Ion-Exchange Chromatography. Tyramine, histamine, putrescine, cadaverine, agmatine, spermidine, and spermine were determined in an extract prepared by blending 25 g of each sample with 50 mL of 7.5% trichloroacetic acid in an Ultraturrax homogenizer (IKA-Werke, Janke & Kunkel, Staufen, Germany) (20000 rpm, 3 min) and centrifuged at 5000*g* for 15 min at 4 °C in a desktop centrifuge (Sorvall RTB6000B, DuPont, Wilmington, DE). The supernatants were filtered through a 0.45 μm Millipore (HVLP) filter, and 10 μL of this filtrate was injected into a HPLC model 1022 with a Pickering PCX 3100 postcolumn system (Pickering Laboratories, Mountain View, CA) following the methodology of Ruiz-Capillas and Moral (*22*). Results are averages of at least three replicates.

Color Measurement. Color, CIE-LAB tristimulus values, originally defined by the Commission Internationale de l'Eclairage (CIE) in 1976 (*23*), lightness, L^*, redness, a^* and yellowness, b^*, of samples were evaluated on a HunterLab model D25-9 (D45/2°) (Hunter Associates Laboratory Inc., Reston, VA). The spectrocolorimeter was calibrated before each series of measurements using a white standard ($L^* = 91.6$; $a^* = -0.8$; $b^* = -1.13$). Eight replicates of the analysis were performed for each formulation.

Statistical Analysis. The results were analyzed statistically using the statistical packages SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). Two-way analysis of variance (ANOVA) was used with time and type of sample as factors, to determine significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Proximate Analysis and pH. The proximate composition of the meat batter was as follows: protein, 16.87 ± 0.02%; moisture, 64.05 ± 0.32%; fat, 13.91 ± 0.21%; and ash, 3.41 ± 0.01%.

The pH changes in the different samples as affected by pressure−temperature combinations and heating treatment are reported in **Figure 1**. The pH of the raw meat batter increased ($P < 0.05$) the effect of pressurization, although this increase did not depend on the pressure temperature ($P > 0.05$). The cooking process caused an increase ($P < 0.05$) of pH in the control sample (C) and in the samples pressurized at 20 °C (HP20) and 40 °C (HP40). Quantitatively this effect was more important in the control sample (**Figure 1**). These results are in line with those reported by Fernández-Martín et al. (*24*) in studies of pressure−heating combinations on pork meat batters.

Irrespective of the treatment assayed, generally pH values did not change ($P > 0.05$) throughout storage. Mean pH values for each sample throughout the storage period were as follows: C, 6.13 ± 0.05; HP7, 6.10 ± 0.04; HP20, 6.14 ± 0.04; HP40, 6.20 ± 0.05. No pH changes during the chilled storage of similar meat products have been reported (*25*, *26*).

Color Parameters. High pressure has been found to increase ($P < 0.05$) the lightness and reduce ($P < 0.05$) the redness and yellowness of meat batters (**Figure 1**). Generally, the changes in yellowness were higher in the raw materials as the pressurization temperature increased (**Figure 1**). Redness and lightness variations were not clearly affected by the pressuriza-

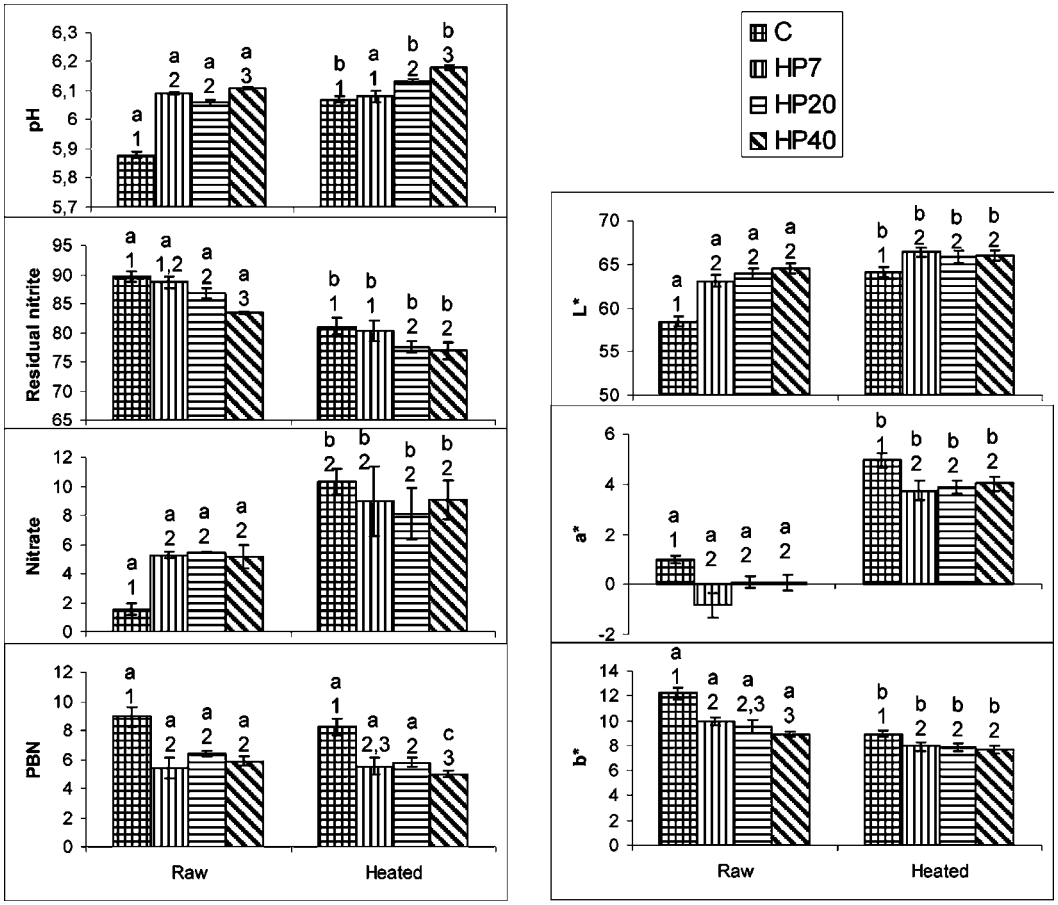

Figure 1. pH, residual nitrite (mg/kg of sample), nitrate (mg/kg of sample), protein-bound nitrite (PBN) (mg/kg, expressed as nitrite equivalents), and color (*L**, *a**, *b**) values of meat batters as affected by high-pressure treatment (HP7, HP20, and HP40, pressurized samples at 300 MPa/10 min at 7, 20, and 40 °C, respectively; C, nonpressurized control sample) and heating process (70 °C/30 min). Different letters for the same sample (raw vs heated) and different numbers between the different raw and heated samples indicate significant differences ($P < 0.05$).

tion conditions. A similar effect of high-pressure treatment on the color parameters has been previously reported (*15*, *27*, *28*).

Generally, the heating process increased ($P < 0.05$) *L** and *a** values (the effect was quantitatively more relevant in redness changes) and decreased ($P < 0.005$) *b** values (**Figure 1**). Irrespective of the treatment, throughout storage no changes ($P > 0.05$) were observed in the color parameters. Mean color parameter values for each sample throughout the storage were as follows: C, $L^* = 63.55 \pm 2.08$, $a^* = 4.41 \pm 1.50$, $b^* = 9.43 \pm 1.24$; HP7, $L^* = 66.29 \pm 1.33$, $a^* = 3.20 \pm 1.79$, $b^* = 8.24 \pm 0.76$; HP20, $L^* = 66.11 \pm 0.93$, $a^* = 3.44 \pm 1.49$, $b^* = 8.14 \pm 0.62$; HP40, $L^* = 66.30 \pm 0.81$, $a^* = 3.41 \pm 1.48$, $b^* = 8.00 \pm 0.43$. Chilled storage in darkness caused minor changes in the color parameters in both nonpressurized (*25*, *29*) and pressurized meat products (*15*).

Residual Nitrite Concentration. The residual nitrite content of raw and nonpressurized meat batter (C) was 89.67 mg/kg (**Figure 1**); this concentration represented almost 60% of the nitrite added. Kolari and Auman (*30*) reported that 20−25% of nitrite has been estimated to disappear during the blending of raw meat mixtures.

Pressure−temperature treatments decreased the residual nitrite levels, and this decrease was greater as the higher pressurization temperature was used. This phenomenon was significant in the HP20 and HP40 samples (**Figure 1**). These results suggest that in the pressure−temperature combinations assayed, the changes in residual nitrite concentration were conditioned more by the temperature than by the pressure applied.

In all of the samples, the cooking process reduced ($P < 0.05$) the residual nitrite levels (**Figure 1**), ranging from 8 to 12% of added nitrite, percentages that were lower than those reported by Hill et al. (*9*) in frankfurters. The effect of the heat treatment in decreasing residual nitrite did not appear to be related to the pressure processing conditions.

Irrespective of the treatment assayed, the residual nitrite levels decreased throughout the chilled storage period. Generally, the HP40 sample exhibited the lowest nitrate content throughout the storage, although this effect was significant in only the last 2 weeks of storage. Residual nitrite depletion during the cooking process and storage of meat products has been extensively described (*9*, *10*, *21*, *25*, *31*, *32*). Residual nitrite depletion during storage is affected by a variety of factors (heat treatment, pH, lean meat content, etc.), following both first- and second-order kinetic equations (*21*, *25*). In this experiment the changes in residual nitrite concentration during storage were fitted to the first-order kinetic equation that yielded a similar constant of nitrite depletion for the four samples (**Table 2**). This indicates that high-pressure processing had no effect on the rate of residual nitrite depletion. The constant residual nitrite depletion established in this experiment was lower than those reported in other experiments (*25*, *33*, *34*).

The residual nitrite levels reported in this study at the end of the storage (about 30% of the nitrite added) (**Table 1**) were similar to those found by other authors (*9*, *26*, *35*, *36*). However, residual nitrite losses during storage larger than those observed in this work have been reported (*10*, *12*, *25*, *34*). These differences could be linked with several factors related to the initial nitrite concentration, intensity of the heating process used,

Table 1. Residual Nitrite, Nitrate, and Protein-Bound Nitrite Contents in the Different Samples throughout the Chilled Storage (2 °C)[a]

	days of storage					
sample	1	6	15	26	40	54
			Residual Nitrite (Milligrams per Kilogram of Sample)			
C	81.06 ± 1.4 a1	74.63 ± 1.1 b1	65.76 ± 0.2 c1	58.85 ± 0.84 d1	52.21 ± 0.25 e1,2	47.09 ± 0.32 f1
HP7	80.25 ± 1.8 a1	74.97 ± 0.8 b1	65.65 ± 0.6 c1	60.97 ± 0.35 d2	52.66 ± 0.45 e1	46.39 ± 0.41 f2
HP20	77.50 ± 1.0 a2	70.30 ± 0.8 b2	64.38 ± 0.9 c2	61.01 ± 1.31 d2	51.34 ± 0.87 e2,3	46.22 ± 0.40 f2
HP40	76.94 ± 1.4 a2	70.12 ± 0.9 b2	64.27 ± 0.8 c2	58.55 ± 0.64 d1	50.85 ± 0.35 e3	44.71 ± 0.13 f3
C	10.34 ± 0.9 a1	6.97 ± 0.8 b1	11.00 ± 0.6 a1	9.98 ± 1.85 a1	12.26 ± 1.80 c1	10.26 ± 0.91 a1
			Nitrate (Milligrams per Kilogram of Sample)			
HP7	8.95 ± 2.4 a1,2	7.31 ± 0.8 b1	8.10 ± 0.7 ab2	6.32 ± 1.04 b2	7.38 ± 1.51 b2	8.51 ± 0.41 a2
HP20	8.09 ± 1.8 a2,3	4.66 ± 0.6 b2	6.94 ± 0.4 c3	3.84 ± 0.95 b3	6.27 ± 0.43 c2	6.67 ± 0.37 c3
HP40	9.06 ± 1.3 a2,3	5.38 ± 1.4 b2	4.80 ± 0.7 b4	5.02 ± 0.86 b3	5.44 ± 0.37 b3	5.54 ± 0.84 b3
			Protein-Bound Nitrite (Milligrams per Kilogram, Expressed as Nitrite Equivalents)			
C	8.24 ± 0.6 a1	9.71 ± 0.1 b1	6.90 ± 0.3 c1	11.42 ± 0.1 d1	11.57 ± 0.2 d1	15.86 ± 1.0 e1
HP7	5.54 ± 0.6 a2	9.39 ± 0.2 b1	6.98 ± 0.5 a1	8.51 ± 0.1 c2	10.04 ± 0.2 d2	13.79 ± 1.2 e2
HP20	5.79 ± 0.3 a2	9.89 ± 0.9 b1	7.04 ± 0.3 c1	9.14 ± 0.5 b2	10.19 ± 0.3 b2	13.20 ± 0.4 d2
HP40	5.01 ± 0.2 a2	9.00 ± 0.5 b1	5.89 ± 0.2 a2	7.62 ± 0.3 c3	8.90 ± 0.1 b3	12.44 ± 0.1 d2

[a] C, nonpressurized control sample; HP7, HP20, and HP40, pressurized samples at 300 MPa/10 min at 7, 20, and 40 °C, respectively, following the heating process (70 °C/30 min). Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

Table 2. Constants of Residual Nitrite Loss (k) over the Chilled Storage

sample	$K \times 10^3$ (days^{-1})	a	$R^{2\,a}$
C	4.4	1.897	0.981
HP7	4.4	1.899	0.991
HP20	4.4	1.880	0.984
HP40	4.3	1.879	0.994

[a] Coefficient of determination for the regression line log[residual nitrite] $= a - kt$, where t is the storage time (days).

absence of reductants, differences in storage time and temperature, meat raw material characteristics, and other differences in processing conditions (*9*, *10*, *13*, *26*, *36*).

No data have been reported on the effect of pressurizing conditions on changes in residual nitrite concentration in meat systems. Karlowski et al. (*15*) studied the residual nitrite content in pressurized (300−600 MPa/10−30 min) loin and ham. No results were shown, but the authors reported that high-pressure treatment after 8 weeks at 4−6 °C did not change the physicochemical characteristics (nitrite) when compared with the initial samples.

Nitrate Content. Nitrate concentration in the raw and nonpressurized sample (C) was 1.54 mg/kg (**Figure 1**), which is indicative of a small conversion of nitrite into nitrate. Pressurization increased ($P < 0.05$) the nitrate content, although this effect did not depend on the pressurization conditions. In all of the samples (nonpressurized and pressurized), the heating process increased ($P < 0.05$) the nitrate concentration, which was proportionally greater ($P < 0.05$) in the nonpressurized product (C) (**Figure 1**). Different authors have highlighted the conversion of nitrite into nitrate in meat products in amounts larger than those observed in this experiment (*10*, *25*, *37*). However, in the absence of reducing agents the level of conversion was considerably lower (*11*).

Generally, over storage and for the C and HP7 samples, nitrate levels did not experience any consistent changes, ranging between 6 and 12 mg/kg (**Table 1**). However, for the HP20 and HP40 samples, nitrate values decreased ($P < 0.05$) during the storage period, generally showing the lowest concentrations. These results suggest that the pressure temperature, although initially it did not seem to affect the conversion of nitrite into nitrate, had an effect throughout storage. It has been reported that nitrate is more stable than nitrite because no changes in nitrate levels occur during the storage of cooked meat products (*35*). However, nitrate content decreasing throughout chilled storage has also been reported (*25*, *35*).

PBN Content. Because a substantial amount of the nitrite added to meat for curing is bound to or reacts with muscle proteins (*10*). The concentration of PBN in uncooked and nonpressurized meat batter (C) was 8.94 mg/kg (**Figure 1**). Pressure treatments decreased ($P < 0.05$) PBN levels in meat batter. Unlike the trend observed for residual nitrite and similar to the changes in nitrate values (**Figure 1**), no effect ($P > 0.05$) was observed in PBN levels due to the pressurization temperature. The heating process had no effect ($P > 0.05$) on the PBN contents in the different samples except in the HP40 sample (**Figure 1**).

The PBN concentration increased ($P < 0.05$) throughout storage in all of the samples (**Table 1**). PBN ranged between 5 and 15 mg/kg (**Table 1**). Generally, the control sample (C) showed the highest PBN concentrations. No clear effect of the pressure−temperature combinations assayed was observed on the PBN content (**Table 1**).

According to Olsman (*33*), the normal levels of this fraction in meat products is about 10−15 mg/kg, very close to the values obtained in this experiment and those reported by several authors (*20*, *21*, *25*). Similarly, increases in PBN levels have been described throughout storage (*21*, *25*). No studies are known that look at the effect of high-pressure processing on PBN.

Detectable Nitrite. Meat products are extremely complex and variable systems that offer an enormous number of constituents that react with nitrite. Consequently, as soon as nitrite is added to meat systems, nitrite depletion begins, so the level of nitrite analytically detectable is greatly reduced and is much less than the initial amount. The rate and extent of nitrite loss is affected by several factors (*10*, *13*, *31*). The amount of nitrite detectable in meat products has been estimated to depend on the reaction of nitrite with different components (*10*, *25*). The amount of nitrite detectable in the form of residual nitrite, nitrate, and PBN (relative to the nitrite added) varied according to the sample type and exhibited the same residual nitrite trend. This behavior was due to the residual nitrite, which was the largest fraction and also the fraction that varies the most (*25*). Percentage changes in this detectable nitrite ranged from values close to 60% (HP20 and HP40) and 66% (C sample) at the beginning

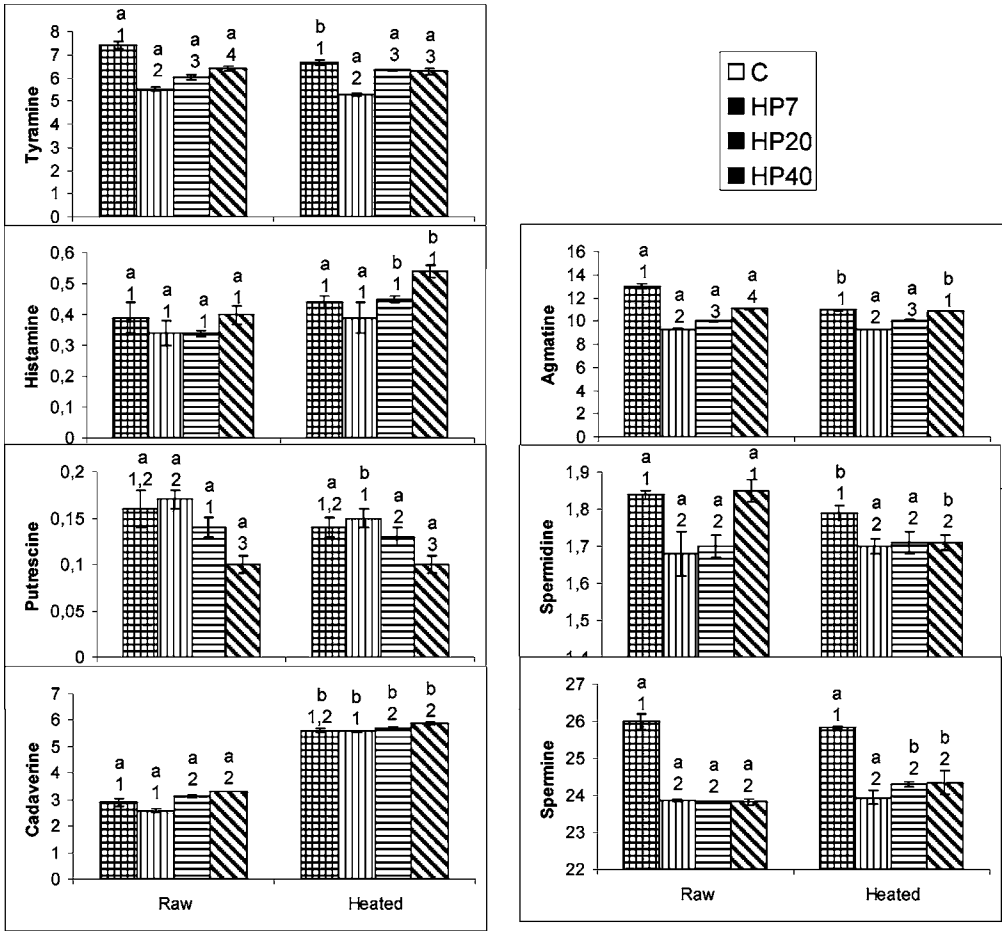

Figure 2. Biogenic amine content (mg/kg) in meat batters as affected by pressure treatment and heating process (C, control sample nonpressurized; HP7, HP20, and HP40, pressurized samples at 300 MPa/30 min at 7, 20, and 40 °C, respectively). Different letters for the same sample (raw vs heated) and different numbers between the different raw and heated samples indicate significant differences ($P < 0.05$).

of storage to 41% (HP40) and 48% (C sample) after 54 days (**Table 1**). On the basis of the same fraction considered in this paper (and also pigment-bound nitrite), other authors (*10*, *25*) reported recoveries of between 28 and 96%.

Biogenic Amines. In the raw and nonpressurized sample (C) spermine was the biogenic amine that exhibited the highest level (**Figure 2**), as is usual in meats and meat products (*6*). High-pressure processing decreased ($P < 0.05$) the concentrations of tyramine, agmatine, and spermine. A similar effect was observed for putrescine and spermidine in some samples (**Figure 2**). In any case, this behavior was not clearly linked with pressurization conditions (**Figure 2**). Some authors (*38*, *39*) have also described decreases in the biogenic amine content in squid mantle or in goat cheeses as an effect of pressure processing.

The cooking process conditioned the biogenic amine content in meat batters differently (**Figure 2**). In both nonpressurized and pressurized samples, heating treatment increased ($P < 0.05$) cadaverine levels. With regard to histamine, the increased concentration was significant only at the highest pressurization temperatures (HP20 and HP40). The cooking process decreased ($P < 0.05$) the agmatine and spermidine contents in the C and HP40 samples (**Figure 2**). Although some authors (*40*) have highlighted a decrease in amine levels from cooking, generally, this fact has been attributed to the effect of amine loss in the exudant, a phenomenon that in this study has not occurred because the samples were placed in closed jars.

In the control sample the tyramine content increased ($P < 0.05$) throughout the storage, reaching higher levels at the end of storage (**Table 3**). The pressure temperature conditioned the tyramine levels that were lower ($P < 0.05$) in the sample treated at 40 °C (HP40) (**Table 3**). In any case, tyramine levels at the end of storage (higher in C) at no time exceeded the limits considered to be toxic for consumers (*41*). No changes were observed in histamine content throughout the storage period (**Table 3**). Histamine content was lower than 1 mg/kg, far from the levels of 100 mg/kg set as toxic by the FDA (*42*). Spermidine and putrescine levels were also very low throughout storage, and any significant changes were observed in these amines (**Table 3**). Although significant, minor increases in spermine concentration were detected in the HP20 and HP40 samples (**Table 3**). Other authors have also reported similar levels in cooked meat products (*6*, *8*, *20*, *43*). Meat is an important source of spermine with physiological effects on the organism (*44*). Cadaverine was the amine that exhibited the most pronounced changes. Cadaverine concentration increased ($P < 0.05$) considerably throughout the storage period, reaching at 54 days and for all of the samples values close to 26 mg/kg (similar to those for spermine). This behavior is not clearly affected by the processing conditions of meat batters (**Table 3**).

Under the experimental conditions the products were very stable, as was shown by the pH level (constant) and low biogenic amine content. Similar results have been described in studies of cooked ham treated with HP and chilled stored (*8*).

The application of high pressure−temperature treatment on different meat products is becoming more important commercially. Its consequences on physicochemical parameters are of great interest because it can also affect the product's

Table 3. Biogenic Amine Content in the Different Samples Throughout the Chilled Storage (2 °C)[a]

	days of storage					
sample	1	6	15	26	40	54
			Tyramine (Milligrams per Kilogram)			
C	6.65 ± 0.11 ab1	6.73 ± 0.15 a1	5.99 ± 0.38 b1,2	8.06 ± 0.20 c1	7.81 ± 0.27 c1	9.82 ± 0.04 d1
HP7	5.27 ± 0.01 a2	6.09 ± 0.05 a2	6.08 ± 0.05 a2	7.07 ± 0.17 b2	7.42 ± 0.27 b1,2	7.38 ± 0.16 b2
HP20	6.33 ± 0.04 a1,3	6.30 ± 0.04 a2	5.89 ± 0.07 b1	6.29 ± 0.28 a3	6.81 ± 0.15 a2	6.11 ± 0.06 ab3
HP40	6.28 ± 0.12 ab3	5.93 ± 0.31 b1,2	6.12 ± 0.12 ab1,2	5.53 ± 0.07 b4	5.66 ± 0.17 b3	5.57 ± 0.08 b4
			Histamine (Milligrams per Kilogram)			
C	0.44 ± 0.02 a1	0.45 ± 0.03 a1	0.43 ± 0.03 a1,2	0.54 ± 0.01 ab1	0.66 ± 0.04 b1	0.47 ± 0.02 ab1
HP7	0.39 ± 0.05 a1	0.45 ± 0.04 a1	0.45 ± 0.01 a1	0.53 ± 0.03 a1	0.42 ± 0.04 a2	0.47 ± 0.01 a1
HP20	0.45 ± 0.01 a1	0.41 ± 0.01 c1	0.50 ± 0.04 abc1,2	0.64 ± 0.13 abc1	0.75 ± 0.10 abc1	0.39 ± 0.02 abc2
HP40	0.54 ± 0.02 a1	0.45 ± 0.04 b1	0.53 ± 0.01 a2	0.57 ± 0.05 a1	0.56 ± 0.04 a1	0.37 ± 0.03 c2
			Putrescine (Milligrams per Kilogram)			
C	0.14 ± 0.01 a1,2	0.19 ± 0.01 b1	0.16 ± 0.01 a1	0.20 ± 0.00 b1	0.24 ± 0.01 b1	0.43 ± 0.01 c1
HP7	0.15 ± 0.01 a1	0.13 ± 0.01 b2	0.13 ± 0.01 b2	0.05 ± 0.00 c2	0.10 ± 0.01 d2	0.16 ± 0.01 a2
HP20	0.13 ± 0.01 a2	0.14 ± 0.01 a2	0.16 ± 0.01 b1,3	0.11 ± 0.02 c3	0.14 ± 0.02 a2	0.14 ± 0.01 a3
HP40	0.10 ± 0.00 a3	0.13 ± 0.00 b2	0.14 ± 0.00 b2,3	0.13 ± 0.01 b3	0.16 ± 0.02 c2	0.11 ± 0.01 b3
			Cadaverine (Milligrams per Kilogram)			
C	5.62 ± 0.07 a1,2	10.22 ± 0.07 b1	14.72 ± 0.22 c1,2	22.00 ± 0.16 d1	23.06 ± 0.29 e1,2	26.17 ± 0.39 f1.2
HP7	5.58 ± 0.02 a1	9.54 ± 0.04 b2	14.01 ± 0.04 c2	16.02 ± 1.52 d2,3	21.42 ± 1.66 e1	26.06 ± 0.19 f1.2
HP20	5.70 ± 0.03 a2	10.18 ± 0.21 b1,2	14.80 ± 0.07 c1	15.97 ± 0.15 d2	23.89 ± 0.28 e2	26.30 ± 0.10 f1
HP40	5.88 ± 0.03 a3	11.45 ± 0.10 b3	13.76 ± 0.06 c3	16.87 ± 0.20 d3	18.89 ± 0.03 e3	25.80 ± 0.18 f2
			Agmatine (Milligrams per Kilogram)			
C	10.97 ± 0.04 a1	10.67 ± 0.11 a1	11.02 ± 0.01 a1	10.69 ± 0.18 a1	11.74 ± 0.19 a1	10.56 ± 0.06 a1
HP7	9.30 ± 0.01 a2	9.11 ± 0.22 a2	9.50 ± 0.34 a2	9.49 ± 0.25 a2	9.33 ± 0.07 a2	9.32 ± 0.10 a2
HP20	10.08 ± 0.05 a3	9.89 ± 0.20 a3	10.05 ± 0.04 a2	10.07 ± 0.05 a2	11.15 ± 0.17 b1	10.11 ± 0.08 a1
HP40	10.89 ± 0.04 a1	11.18 ± 0.08 ab4	11.78 ± 0.23 b1	11.33 ± 0.09 ab3	10.95 ± 0.02 a1	11.33 ± 0.13 ab4
			Spermidine (Milligrams per Kilogram)			
C	1.79 ± 0.02 a1	1.84 ± 0.06 a1	1.82 ± 0.02 a1	2.20 ± 0.04 b1	1.92 ± 0.05 ab1	2.41 ± 0.14 b1
HP7	1.70 ± 0.03 a2	1.70 ± 0.17 a1	1.79 ± 0.10 a1,2	1.73 ± 0.06 a2	1.81 ± 0.06 ab1	1.90 ± 0.07 b2
HP20	1.71 ± 0.03 a2	1.70 ± 0.05 ab1	1.73 ± 0.01 a2	1.76 ± 0.06 ab2	1.89 ± 0.10 ab1	1.80 ± 0.00 ab2
HP40	1.71 ± 0.02 a2	1.75 ± 0.05 a1	1.79 ± 0.07 ab1,2	1.78 ± 0.11 ab2	1.85 ± 0.04 b1	1.85 ± 0.04 b2
			Spermine (Milligrams per Kilogram)			
C	25.82 ± 0.03 a1	26.44 ± 1.12 a1,2	25.99 ± 0.23 ab1,2	26.68±0.31 a1	27.14 ± 0.97 a1	27.81 ± 1.85 a1
HP7	23.95 ± 0.18 a2	26.62 ± 0.18 b1	24.17 ± 0.17 a1	25.58 ± 0.81 ab1,3	25.61 ± 0.43 ab1	26.98 ± 0.72 ab1
HP20	24.30 ± 0.08 a2	25.35 ± 0.44 ab2	25.44 ± 0.33 ab2	24.15 ± 0.13 a2,3	27.40 ± 1.31 bc1	26.58 ± 0.19 c1
HP40	24.34 ± 0.32 a2	25.98 ± 0.28 b1,2	25.52 ± 0.21 ab2	25.30 ± 0.50 ab2,3	26.47 ± 0.09 bc1	26.89 ± 0.60 c1

[a] C, nonpressurized control sample; HP7, HP20, and HP40, pressurized samples at 300 MPa/10 min at 7, 20, and 40 °C, respectively, following the heating process (70 °C/30 min). Different letters in the same row and different numbers in the same column indicate significant differences ($P < 0.05$).

characteristics and have implications for some aspects related to consumers' health. This is the case of biogenic amine formation and nitrite reactions.

The results of this experiment showed that pressurization conditions hardly affect biogenic amine formation and residual nitrite depletion throughout the storage. Nitrate conversion and protein-bound nitrite were dependent on the pressure−temperature combinations, especially the pressurization temperature used. Biogenic amine concentration levels were below the limits allowed.

SAFETY

The chemicals, equipment, and procedures of this study were handled and performed in accordance with usual precautionary measures.

ABBREVIATIONS USED

MPa, megapascal; min, minutes; FAAs, free amino acids; kg, kilogram; mm, millimeters; STP, sodium tripolyphosphate; FIA, flow injection analysis; NO_2, nitite; NO_3, nitrate; PBN, protein-bound nitrite; CIE, Commission Internationale de L'Eclairage; *L**, lightness; *a**, redness; *b**, yellowness; FDA, U.S. Food and Drug Administration.

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