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Consequences of high-pressure processing of vacuum-packaged frankfurters on the formation of polyamines: Effect of chilled storage

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Abstract

The effect of high-pressure processing (400 MPa/10 min/30 °C) (HPP) and thermal superficial pasteurization treatment (SPT) on the formation of polyamines, microorganism inactivation and physico-chemical characteristics in vacuum-packaged frankfurters was studied. The consequences of these treatments were also evaluated throughout chilled storage (2 °C) for up to 141 days. Generally, the formation of polyamines was affected by vacuum-packaged frankfurter processing conditions and depended on the type of amine. Tyramine, putrescine and cadaverine were the amines that exhibited the greatest changes ($P < 0.05$) throughout storage, although no changes ($P > 0.05$) were detected in the pressurized sample. Both superficial pasteurization (SPT sample) and pressurization (HPP sample) caused decreases in the levels of total viable and lactic acid bacteria counts. Microbial inactivation was higher in the pressurized samples, which even after 141 days of storage the microbial population was lower than 3 log cfu/g. Irrespective of the treatments assayed, a decrease ($P < 0.05$) was observed in hardness and chewiness throughout storage.

Keywords: Frankfurter; Vacuum-packaged; High pressure; Polyamines; Microbiota; Chilled storage

1. Introduction

Society is increasingly aware of the importance of diet for health. Hence, any issue related to food safety has a considerable impact on consumer behaviour and official policy. It is known that the consumption of products with high concentrations of polyamines may have a toxicological risk for the consumer (Bardócz, 1995; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). These compounds are found in varying concentrations in a wide range of foods, including many meat products (Ruiz-Capillas & Jiménez-Colmenero, 2004a). Polyamines are primarily formed by decarboxylation of free amino acids (FAAs) from the decarboxylase action of the amino acid enzymes. Factors associated with the raw material (meat composition, pH, handling conditions, etc.) as well as the substrate source and reaction medium directly affect the availability of FAAs, whereas the presence of the enzyme is closely linked to microbiological aspects (bacterial species, strain and bacterial growth, etc.). (Bardócz, 1995; Halász et al., 1994; Ruiz-Capillas & Jiménez-Colmenero, 2004b). These factors are obviously interdependent and are further affected by the technological processes associated with the types of meat derivative, processing and storage conditions.

In recent years the meat industry has been applying emerging technologies like high pressure as a new preservation process due to their effect on microorganisms. It is a non-thermal method that causes microbial inactivation and the extent of its effect depends on a number of factors related to pressurization conditions (pressure levels, process temperature and time, etc.), the microorganism itself (type or phase of growth) and certain characteristics of the food system (composition, pH, water activity, etc.). High-pressure processing has been identified as especially interesting for some meat products to use in the final

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preservation step. Even in good hygienic conditions, these products are recontaminated due to the handling process (portioning, slicing or comminuting) after thermal treatment, and this has a direct impact on their shelf life and safety (Carpi et al., 1999; Hayman, Baxter, O'Riordan, & Stewart, 2004; López-Caballero, Carballo, & Jiménez-Colmenero, 1999; López-Caballero, Carballo, & Jiménez-Colmenero, 2002; Yuste, Mor-Mur, Guamis, & Pla, 1999). Such is the case of meat products like frankfurters where the removal of the casing and the packaging process generate some contamination (Yuste, Pla, Capellas, Ponce, & Mor-Mur, 2000). Thus, subsequent treatment is necessary to guarantee a satisfactory refrigerated shelf life. With that purpose, high-pressure-moderate temperature "pasteurization" has been described as an effective preservation method that can replace heat pasteurization applied to some cooked products after packaging (Cheftel & Culioli, 1997).

Combined high pressure (200–600 MPa) – temperatures (2–80 °C) have been assayed to reduce the microbial population in various cooked meat products (Carpi et al., 1999; Garriga, Grébol, Aymerich, Monfort, & Hugas, 2004; Hayman et al., 2004; López-Caballero et al., 1999; López-Caballero et al., 2002; Patterson, 2005; Yuste et al., 1999; Yuste et al., 2000). However, such pressurization conditions cannot prevent the recovery of microorganisms and their subsequent ability to develop and perform their usual metabolic activity, which can obviously affect the formation of biogenic amines or polyamines during chilled storage. Different concentrations of biogenic amines in retail pressurized meat products have been reported (Ruiz-Capillas & Jiménez-Colmenero, 2004a). Ruiz-Capillas, Carballo, and Jiménez-Colmenero (2006) reported changes in biogenic amine content during chilled storage in pressurized (400 MPa/10 min/30 °C) vacuum-packaged cooked sliced ham.

As far as the authors know, no studies have been done to evaluate the formation of biogenic amines or polyamines in pressurized frankfurter sausage during chilled storage. This fact must be known to avoid the negative implications of this kind of processed meat product for consumers. The purpose of this study was to analyze how the application of high pressure (400 MPa/10 min/30 °C) and thermal superficial pasteurization affect the formation of polyamines, microorganism inactivation and the physico-chemical characteristics of vacuum-packaged frankfurters, and also the consequences of these treatments throughout chilled storage (2 °C).

2. Materials and methods

2.1. Preparation of frankfurters

Fresh post-rigor pork leg meat (mixture of *M. biceps femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. gracilis* and *M. adductor*) and pork fat were obtained from a local meat market. The meat was trimmed of fat and connective tissue and the pork fat was passed through a grinder with a 0.6 mm plate. Lots of approx. 1 kg were vacuum packed, frozen and stored at −20 °C until use, which took place within 3 weeks.

Meat and fat packages were thawed (approx. 18 h at 3 ± 2 °C) prior to use. The preparation of meat batters were as described by Jiménez Colmenero, Barreto, Mota, and Carballo (1995). Pork meat, back fat and water were combined with sodium chloride (2.5%), sodium tripolyphosphate 0.18% and sodium nitrite 150 ppm (Panreac Quimica, S.A. Barcelona, Spain). The meat batter was stuffed into 20 mm diameter Nojax cellulose casings (Viscase S.A., Bagnold Cedex, Francia) and hand-linked. The frankfurters were heat processed in a force air oven (Rational, combi-master CM6, Spain) until the internal temperature reached 70 °C. Heat processing conditions were established beforehand, and the internal temperature was monitored throughout heating by means of thermocouples inserted in each frankfurter (thermal centre) and connected to a temperature recorder (Yokogawa Hokuskin Electric YEM, Mod. 3087, Tokyo, Japan). Then the frankfurters were cooled, their casings removed, and vacuum packed (4 frankfurters per package) in plastic bags (Wipak® Pae 110KFP, oxygen permeability of 30 cm^3/m^2/24 h to 23 °C, RH −75% and 1 bar).

2.2. Pressure and thermal treatments of vacuum-packaged frankfurters

The vacuum-packaged frankfurters were divided into three lots. One of these lots was high-pressure processing (HPP) at 400 MPa/10 min/30 °C as reported by Ruiz-Capillas et al. (in press). The second one was subjected to a superficial thermal pasteurization (STP) process by immersion in a boiling water bath for 2 min. The third lot, not subjected to pasteurization treatment was the control sample (C). The three types of samples were stored in a chilled room at 2 °C (±1 °C) during storage for 62 days in control frankfurters and for 141 days in pasteurized sausages.

2.3. Proximate analysis

Moisture and ash content of the frankfurters were determined (AOAC, 2000) in quadruplicate. Fat content was evaluated (in duplicate) according to Bligh and Dyer (1959). Protein content was measured in quadruplicate by a Nitrogen Determinator LECO FP-2000 (Leco Corporation, St Joseph, MI).

2.4. pH

The pH was determined on a Radiometer model PHM 93 pH-meter (Meterlab, Copenhagen, Denmark) at room temperature on homogenates of meat products in water in a ratio 1:10 (w/v). Six replicates of the analysis were performed for each formulation.

2.5. Colour measurement

Colour, CIE-LAB triestimulus values, 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). Eight replicates of the analysis were performed from 2 bags of each formulation in each day of analysis; these samples were also used for the texture analysis.

2.6. Texture profile analysis

Texture Profile Analysis (TPA) was performed in an Instron machine (model 4501, Instron Engineering Corp., Canton, M.A.) as described by Bourne (1978). Six cores (diam. = 20 mm, height = 25 mm) of cooked samples were axially compressed to 40% of their original height. Force-time deformation curves were derived with a 5 kN load cell, applied at a crosshead speed of 50 mm/min. Attributes were calculated as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first compression; chewiness (Cw) = Hd × Ch × Sp ($N \times$ mm). Measurement of samples was carried out at room temperature.

2.7. Microbiological analysis

Ten grams of each sample (from 2 plastic bags of each formulation) was taken and placed in a sterile plastic bag with 90 ml of peptone water (0.1%) with 0.85% NaCl. After 2 min in a stomacher blender (Stomacher Colworth 400, Seward, UK), appropriate decimal dilutions were pour-plated (1 ml) on the following media: Plate Count Agar (PCA) (Merck, Germany) for total viable count (TVC) (30 °C for 72 h); De Man, Rogosa, Sharp Agar (MRS) (Merck, Germany) for lactic acid bacteria (LAB) (30 °C for 3–5 days); and Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony forming units per gram (log cfu/g).

2.8. Analysis of polyamines by ion-exchange chromatography

Tyramine, histamine, putrescine, cadaverine, agmatine, spermidine and spermine were determined following the methodology of Ruiz-Capillas and Moral (2001) in a HPLC model 1022 with a Pickering PCX 3100 post-column system (Pickering Laboratories, Mountain View, Ca, USA). The repetitiveness of the method was below 5%. The detection limit and determination limit were below 0.05–0.08 mg/l and 0.2 mg/l, respectively. Results are averages of at least 3 replicates; the sample use for this determination was the same as use for the microbiology.

2.9. Statistical analysis

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

3. Results and discussion

3.1. General

The proximate analysis of the frankfurter used in this study was: moisture, 63.82 ± 0.19%; protein, 16.98 ± 0.05%; fat 15.35 ± 0.21% and ash 2.86 ± 0.05% content.

3.2. Effect of vacuum-packaged frankfurter processing

Both pressurization and superficial thermal treatment affected the frankfurter characteristics (Tables 1–5). It is known that the effect of applying high pressures on foods depends on several factors, among them those that refer to both meat system conditions (raw or heated) and pressure/temperature combinations (Jiménez-Colmenero, 2002). Although numerous studies exist for evaluating the consequences of the pressurization of raw muscle systems (pressurized at low and constant temperature or heated under high pressure conditions), there are fewer studies where pressurization (at low or mild temperatures) is applied on cooked meat products. In such cases, it must be taken into account that heat-induced changes prior to high-pressure processing influence the pressure effect on the meat system (Jiménez-Colmenero, 2002).

The microbial population of the control sample (C) showed that vacuum- packaged frankfurters exhibited some contamination (Table 1) compatible with the handling process prior to the packaging of cooked sausages (Yuste et al., 2000). This flora consisted mainly of lactic acid bacteria, typical spoilage microbiota of vacuum-packaged emulsion type meat products (Yuste et al., 2000). The inactivation of spoilage microorganisms through different treatments after frankfurters are packaged is essential to improve their microbial quality. Vacuum-packaged frankfurter processing affected the microbial population of the products (Table 1). Both superficial pasteurization (SPT sample) and pressurization (HPP sample) treatments caused decreases in TVC and LAB levels. In the three samples studied the *Enterobacteriaceae* levels were lower than 1 log cfu/g. The most pronounced decreases in TVC and LAB were observed in the vacuum-packaged frankfurters treated with high pressures (HPP).

There are numerous studies on the effect of pressurization on microorganisms in different muscle-based products, among them, studies concerning the application of pressurization processes on cooked meat products (Carpi et al., 1999; Garriga et al., 2004; Hayman et al., 2004; López-Caballero et al., 1999; López-Caballero et al., 2002;

Table 1
Microorganism counts (log cfu/g) in samples as affected by vacuum-packaged frankfurter processing (C: control, no pasteurization process applied; SPT: superficial pasteurization treatment in boiling water during 2 min, HPP: high-pressure processing at 400 MPa/10 min/30 °C), and chilled storage (2 °C)

Microorganisms	Samples	Days of storage						
		0	13	27	48	62	114	141
Total viable counts	C	4.63 ± 0.08	4.81 ± 0.13	4.60 ± 0.02	6.01 ± 0.05	6.24 ± 0.02		
	SPT	3.36 ± 0.12	3.47 ± 0.61	3.00 ± 0.05	3.22 ± 0.11	3.59 ± 0.09	4.31 ± 0.02	6.90 ± 0.60
	HPP	2.47 ± 0.08	2.95 ± 0.07	2.50 ± 0.70	2.15 ± 0.21	2.15 ± 0.15	2.10 ± 0.26	2.80 ± 0.28
Lactic acid bacteria	C	4.27 ± 0.02	4.66 ± 0.08	4.52 ± 0.11	6.01 ± 0.04	5.57 ± 0.07		
	SPT	3.15 ± 0.15	3.00 ± 0.09	3.50 ± 0.22	2.15 ± 0.21	3.59 ± 0.02	4.13 ± 0.06	6.29 ± 0.02
	HPP	2.19 ± 0.06	2.50 ± 0.71	1.80 ± 0.04	2.00 ± 0.03	2.15 ± 0.15	2.08 ± 0.01	2.50 ± 0.71
Enterobacteriaceae	C	<1	1.15 ± 0.21	<1	<1	<1		
	SPT	<1	<1	<1	<1	<1	<1	<1
	HPP	<1	<1	<1	<1	<1	<1	<1

Table 2
Polyamines content (mg/kg) in samples as affected by vacuum-packaged frankfurter processing (C: control, no pasteurization process applied; SPT: superficial pasteurization treatment in boiling water during 2 min, HPP: high-pressure processing at 400 MPa/10 min/30 °C), and chilled storage (2 °C)

Polyamines	Samples	Days of storage						
		0	13	27	48	62	114	141
Tyramine	C	6.1a1	6.9a,b1	7.3b1	11.5c1	14.3d1		
	SPT	6.5a1	5.5a1	5.2a2	5.8a2	7.9a,b2	9.3b1	32.1c1
	HPP	6.8a,b1	5.7a1	6.7a,b1,2	7.1a,b3	5.9a3	6.1a2	7.5b2
Histamine	C	1.5a1	1.9a1	0.5b1	0.4b1	0.7b1		
	SPT	1.7a1	1.4a,b1	1.1b,c2	0.8c,d1	0.6d1	0.4d1	0.5d1
	HPP	2.1a2	1.3b1	0.8c1,2	0.7c1	0.5c1	0.6c1	0.4c1
Putrescine	C	0.1a1	0.4b1	0.3b,1	1.0c1	1.5c1		
	SPT	0.2a1	0.3b1	0.5b2	0.3b2	0.4b2	0.1a1	2.1c1
	HPP	0.2a1	0.3b1	0.2a,b1	0.4b2	0.5b2	0.3b2	0.4b2
Cadaverine	C	3.9a1	4.1a1	4.1a1	5.1a,b1	6.1b1		
	SPT	4.0a1	4.2a1	4.1a1	3.9a2	3.9a2	3.9a1	8.2b1
	HPP	4.3a2	3.9a1	4.4a1	4.0a1,2	4.1a2	3.5a1	3.9a2
Agmatine	C	25.2a1	25.5a1	25.9a1,2	24.7a1	25.1a1		
	SPT	25.9a1	25.6a1	25.7a1	25.6a2	24.9a2	23.7b1	23.7b1
	HPP	26.9a2	24.1a,b2	26.7a2	25.4a,b2	25.2b1	23.9b1	23.8b1
Spermidine	C	1.3a1	1.4a1	1.4a1	1.3a1	1.3a1		
	SPT	1.4a1	1.5a1	1.4a1	1.3a1	1.3a1	1.3a1	1.3a1
	HPP	1.5a1	1.3a1	1.5a1	1.3a1	1.3a1	1.1a1	1.1a1
Spermine	C	24.2a,b1	25.6a1	25.5a1	23.9b1	23.9b1		
	SPT	25.1a2	25.7a1	25.1a1	24.1a1	22.9b2	22.5b1	21.6b1
	HPP	27.7a3	24.1b2	26.8a2	24.0b1	23.6b,c1	22.3c1	22.6c1

Different letters in the same row and different number in the same column (for the same amine) indicate significant differences ($P < 0.05$).

Table 3
pH changes in samples as affected by vacuum-packaged frankfurter processing (C: control, no pasteurization process applied; SPT: superficial pasteurization treatment in boiling water during 2 min, HPP: high-pressure processing at 400 MPa/10 min/30 °C), and chilled storage (2 °C)

Samples	Days of storage						
	0	13	27	48	62	114	141
C	6.44a1	6.19b1,2	6.11b,c1	6.13c1	5.97d1		
SPT	6.34a1,2	6.18b1	6.16b1	6.17b1	6.03c1,2	5.94c,d1	5.82d1
HPP	6.28a2	6.23a,b2	6.31a2	6.20b2	6.08c2	6.02c,d1	5.93d1

Different letters in the same row and different number in the same column indicate significant differences ($P < 0.05$).

Karlowski et al., 2002; Yuste et al., 1999; Yuste et al., 2000). Along with aspects related to the microorganisms themselves, both pressure conditions (among others, pressurization temperature) and the nature of medium (the cooking process has a marked and permanent effect on this) condition the pressure inactivation effect (Cheftel & Culioli, 1997; Patterson, 2005). According with our results, the pressure (400 MPa/20 min/7 °C) reduction of TVC and LAB in

Table 4
Texture parameters variations in samples as affected by vacuum-packaged frankfurter processing (C: control, no pasteurization process applied; SPT: superficial pasteurization treatment in boiling water during 2 min, HPP: high-pressure processing at 400 MPa/10 min/30 °C), and chilled storage (2 °C)

Parameters	Samples	Days of storage		
		0	27	62
Hardness (*N*)	C	23.4a1	22.8a1	19.9b1
	SPT	24.7a1	22.7b1	20.5c1
	HPP	20.6a2	18.0b2	16.1c2
Springiness (mm)	C	6.89a1	6.64b1	6.71a,b1
	SPT	6.67a1	6.58a1	6.71a1
	HPP	6.81a1	6.62a1	6.68a1
Cohesiveness	C	0.59a1	0.60a1	0.62a1
	SPT	0.58a1	0.59a1	0.60a1
	HPP	0.61a1	0.61a1	0.63a1
Chewiness (N × mm)	C	94.69a1	89.99a,b1	81.94b1
	SPT	95.42a1	88.38a,b1	82.64b1
	HPP	85.50a2	72.64b2	67.25b2

Different letters in the same row and different number in the same column indicate significant differences ($P < 0.05$).

Table 5
Colour parameters variations in samples as affected by vacuum-packaged frankfurter processing (C: control, no pasteurization process applied; SPT: superficial pasteurization treatment in boiling water during 2 min, HPP: high-pressure processing at 400 MPa/10 min/30 °C), and chilled storage (2 °C)

Parameters	Samples	Days of storage		
		0	27	62
Lightness (L^*)	C	69.47a1	69.24a1	69.19a1
	SPT	70.43a1	69.77a1	69.95a1
	HPP	69.32a1	69.95a1	70.15a1
Redness (a^*)	C	5.79a1	5.51a,1	5.55a1
	SPT	6.44a2	6.05a1	6.14a2
	HPP	6.05a1,2	6.05a1	6.09a1,2
Yellowness (b^*)	C	8.73a1	9.13a1	9.04a1
	SPT	9.00a1,2	9.06a1	9.03a1
	HPP	9.29a2	9.01a1	9.02a1

Different letters in the same row and different number in the same column (for the same parameters) indicate significant differences ($P < 0.05$).

sliced cooked ham has been reported (López-Caballero et al., 1999). Yuste et al. (1999) did not observe microbial inactivation in cooked poultry sausages when pressurization (500 MPa/5–15 min) was applied at temperatures lower than 50 °C. However, the pressurization of cooked sausages at higher temperatures (65–80 °C) generated great inactivation of psychtrophs, mesophile, lactic acid bacteria and *enterobacteriaceae* (Yuste et al., 1999; Yuste et al., 2000). The application of 300–600 MPa/10–30 min to cooked pork ham led to a reduction of the total count of microorganisms (Karlowski et al., 2002).

Vacuum-packaged frankfurter processing conditioned the polyamines content of products (Table 2). While superficial pasteurization treatment did not have any effect on polyamines levels (except for spermine), in the sample subjected to high-pressure processing (HPP) a slight, yet significant increase in histamine, cadaverine, agmatine and spermine levels was detected (Table 2). Unlike in observed in this study, the pressurization (400 MPa/10 min/30 °C) of vacuum-packaged sliced cooked ham increased tyramine levels, whereas no changes were reported in other polyamines (Ruiz-Capillas et al., in press).

The pressurization process caused a decrease ($P < 0.05$) in pH in relation to the control sample (C). However, no differences ($P > 0.05$) were observed between the SPT and HPP samples (Table 3). No changes in pH as a result of pressurization of the pre-packaged sliced cooked ham or various ready-to-eat (cooked) meat products were found by other authors ((Hayman et al., 2004; López-Caballero et al., 1999).

High-pressure processing of frankfurters caused a significant decrease in hardness and chewiness in comparison with the non-pressurized samples (Table 4). The different treatments did not affect the springiness and cohesiveness ($P > 0.05$). Mor-Mur & Yuste (2003) treated vacuum-packaged cooked sausages at 500 MPa/5 or 15 min/65 °C, and they compared their behaviour with that of sausages treated with heat pasteurization (80–85 °C for 40 min). They reported that pressurized sausages were more cohesive and less firm (hard) than heat-treated sausages. Karlowski et al. (2002) reported that the processing of cooked ham at 400 MPa/10 min/room temperature had no effect on the penetration force of the product.

Thermal superficial treatment and pressure treatment increased ($P < 0.05$) redness and yellowness, respectively (Table 5). Although pressure-induced changes have been reported in the high-pressure processing of raw muscle systems or muscle systems cooked under pressure, colour is not affected by pressure when the products previously treated by heat, as is the case of cooked ham (Carpi et al., 1999; López-Caballero et al., 2002; Karlowski et al., 2002), or cooked sausages (Mor-Mur & Yuste, 2003). This behaviour has been associated with thermal induced changes (in the presence of nitrite) in the product. The resistance to colour changes makes this kind of meat products good candidates for high-pressure processing (Cheftel & Culioli, 1997).

3.3. Effect of chilled storage

The microbial population of samples throughout the storage period was affected by both vacuum-packaged frankfurter procedures: thermal superficial pasteurization and pressure treatments (Table 1). Throughout storage, the control sample exhibited the highest TVC and LAB counts, which did not changed until day 48 when levels of 6.01 log cfu/g were reached (Table 1). Given that these levels have been described as the limit for rejection of the product (Borch, Nerbrink, & Svemsson, 1988), at 62 days the storage finished for the control sample (C). For the other two samples (HPP and SPT), it was thought interest-

ing to extend the study for a longer period of time to evaluate the effect of treatments applied on vacuum-packaged frankfurter to limited the recontamination effect. In this sense, levels in the region of 6 log cfu/g were not reached in the SPT sample until 141 days of storage, more than 90 days after the sample C. The effectiveness of high-pressure processing was even greater, since the microbial populations in the HPP sample remained very low throughout storage. Even after 141 days, both the TVC and LAB counts were lower than 3 log cfu/g (Table 1).

Different studies have been done to evaluate the effect of pressure treatment on the chilled storage stability of cooked meat products. Pressurization (600 MPa/20 °C/3 min) can extend the refrigerated (4 °C) shelf life of various cooked (comminuted and whole) beef products (Hayman et al., 2004). Karlowski et al. (2002) highlight that while 300–400 MPa pressure applied for 10 min was insufficient to extend the storage period of cooked pork ham, the treatment was effective at 500–600 MPa. High-pressure treatment at 600 MPa for 6 min at 31 °C extended the storage period (at 4 °C) of cooked ham for at least 30 days compared with the non-treated sample (Garriga et al., 2004). In the same conditions assayed in this experiment Ruiz-Capillas et al. (in press) observed that high pressure increased the shelf life (2 °C) of the vacuum-packaged cooked sliced ham (compared with non-pressurized) by at least 35 days. Yuste et al. (2000) reported that heat (80–85 °C/40 min) and pressure treatments (500 MPa/5–15 min/65 °C) of cooked sausages cause sublethal injury to the bacterial cells, which can be recovered during storage. Although this phenomenon has been described in other pressurized cooked meat products (Carpi et al., 1999; Garriga et al., 2004; López-Caballero et al., 1999; López-Caballero et al., 2002), in this study, the recovery and subsequent ability to develop TVC and lactic acid bacteria was not observed (Table 1). These results indicate that combined high-pressure-moderate temperature was more effective than thermal superficial pasteurization in delaying spoilage and extending the shelf life of refrigerated vacuum-packaged frankfurters. Obviously, the application of higher pasteurization thermal treatments (80–85 °C for 40 min after the packaging of cooked sausage) than those assayed in this study have to provide greater microbial stability (Yuste et al., 1999).

Generally, the changes in the concentration of polyamines throughout storage varied depending on the vacuum-packaged frankfurter processing conditions and type of amine (Table 2). Irrespective of the treatment assayed, during the first two months of storage, histamine level decreased and agmatine, spermidine and spermine levels remained constant (Table 2). A different behaviour was observed in sample C where an increase ($P < 0.05$) was detected in putrescine and cadaverine, and especially tyramine levels that were double their initial value at 48 days of storage (Table 2). In the SPT sample, a storage longer than 62 days increased ($P < 0.05$) of tyramine, putrescine and cadaverine contents (Table 2). The increase in these polyamines matched the increase observed in TVC and LAB counts (Table 1), responsible for the formation of these amines (Edwards, Dainty, Hibard, & Ramantanis, 1987; Halász et al., 1994). Nevertheless, the ability of LAB to degrade amino acids greately varies among species. (Fernández & Zúñiga, 2006). Increases in tyramine and putrescine levels during different chilled storage conditions have been reported in pressurized vacuum-packaged cooked sliced ham (Ruiz-Capillas et al., in press). Although cadaverine and putrescine are related to product spoilage (Edwards et al., 1987; Halász et al., 1994), putrescine levels are very low for cooked products like cooked sliced ham (Ruiz-Capillas & Jiménez-Colmenero, 2004a; Ruiz-Capillas et al., 2006) or frankfurter (Table 2). Histamine levels were also low, a fact that has been highlighted by other authors in this kind of products (Halász et al., 1994; Hernández-Jover, Izquierdo-Pulido, Veciana-Nogues, & Vidal-Carou, 1996; Ruiz-Capillas & Jiménez-Colmenero, 2004a). As previously observed above, this study also detected that the pressurization of cooked meat product has a greater effect on the presence of cadaverine and tyramine than on the rest of the amines (Ruiz-Capillas et al., in press).

In all the samples studied, a decrease ($P < 0.05$) was detected in pH levels (Table 3) throughout storage, which was more pronounced in the control sample. This behaviour throughout storage was associated with microbial growth, primarily of lactic bacteria that produce lactic acid responsible for a decrease in pH (Samelis, Kakouri, Georgiadou, & Metaxopoulos, 1998). Similar trends in pH decreases in studies on cooked ham treated with high pressures have been observed previously (Ruiz-Capillas et al., in press).

Irrespective of the treatments assayed, a decrease was observed in hardness and chewiness (Table 4) throughout storage, which was quantitatively similar in all the samples. Pressurized vacuum-packaged frankfurter (HPP) exhibited the lowest Hd and Cw values. For all the samples, no differences ($P > 0.05$) were observed in the cohesiveness or springiness values throughout chilled storage (Table 4). Different results as to the effect of the chilled storage on the textural properties of high-pressure cooked meat products have been described. Similary to this experiment Cofrades, Carballo, & Jiménez Colmenero (1997) reported decreases in Hd and Cw in high- and low-fat frankfurters during chilled storage. Carpi et al. (1999) reported that the shear force increased in both non-pressurized and pressurized sliced cooked ham during chilled storage. However, the physico-chemical characteristics (texture, colour, etc.) of cooked ham treated at 300–600 MPa/10–30 min/room temperature did not change throughout the chilled storage period (8 weeks at 4–6 °C) (Karlowski et al., 2002).

Generally, no changes were detected in the colour parameters over storage (Table 5). Similar results have been reported by Carpi et al. (1999).

The results obtained in this study reveal that high-pressure processing is a more effective procedure than thermal

superficial pasteurization for reducing the effects of recontamination as well as their consequences on the spoilage processes that these products experience throughout storage. The effect of reduced recontamination was evident from less microbial development and the formation of some polyamines, and this effect was even more pronounced for long storage periods.

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