

Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life

M. Garriga^{a,*}, N. Grèbol^a, M.T. Aymerich^a, J.M. Monfort^a, M. Hugas^b

^aIRTA-Meat Technology Center, Granja Camps i Armet s/n. 17121 Monells, Spain

^bEuropean Food Safety Authority (EFSA), 10 rue de Genève, B-1040 Brussels, Belgium

Received 9 January 2004; accepted 1 July 2004

Abstract

The behaviour of spoilage and pathogenic microorganisms was evaluated after high-pressure treatment (600 MPa 6 min, 31 °C) and during chilled storage at 4 °C for up to 120 days of commercial meat products. The objective was to determine if this pressure treatment is a valid process to reduce the safety risks associated with *Salmonella* and *Listeria monocytogenes*, and if it effectively avoids or delays the growth of spoilage microorganisms during the chilled storage time evaluated. The meat products covered by this study were cooked meat products (sliced cooked ham, pH 6.25, a_w 0.978), dry cured meat products (sliced dry cured ham, pH 5.81, a_w 0.890), and raw marinated meats (sliced marinated beef loin, pH 5.88, a_w 0.985). HPP at 600 MPa for 6 min was an efficient method for avoiding the growth of yeasts and Enterobacteriaceae with a potential to produce off-flavours and for delaying the growth of lactic acid bacteria as spoilage microorganisms. HPP reduced the safety risks associated with *Salmonella* and *L. monocytogenes* in sliced marinated beef loin.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: High-pressure processing; Meat products; Microbial inactivation; Shelf life

Industrial relevance: High pressure is a preservation technique which seems a natural choice for meat and meat products. However, it has taken quite a while until products treated at an industrial level are appearing. The study aimed at cooked and dry cured ham and at marinated beef loin and the evaluation of microbial growth during subsequent extended chilled storage. Clearly high pressure treatment reduced microbial risks over non treated products and the authors rightly point to the composition of the products as one key factor influencing the effectiveness of high pressure processing.

1. Introduction

Recent food safety crises (BSE, dioxines, food poisoning outbreaks) have alarmed consumers who require wholesome meat products with minimal processing and with a “fresh” appearance. To combine these demands without compromising safety, it is necessary to implement new preservation technologies.

High-pressure processing (HPP) is an attractive preservation technology that is mild for food but

eliminates pathogenic and spoilage microorganisms; it has a good potential for the meat industry in particular. HPP at low or moderate temperature causes inactivation of certain enzymes and the destruction of microbial vegetative cells without changing, in general, the sensorial attributes of the product. However, the resistance of the microorganisms is variable depending on the strain and the meat matrix to be treated. The efficacy of the treatment also depends on the achieved pressure and on the exposure time.

In 1899, Hite and his researchers were the first to report microbial inactivation after pressure treatments, but the interest in this technology was not renewed until the 1980s, when Farkas, at the University of Delaware

* Corresponding author. Tel.: +34 972 630052; fax +34 972630373.

E-mail address: margarita.garriga@irta.es (M. Garriga).

(USA), showed that high-pressure processing reduced the microbiological load and the enzymatic activity of foods, whereas many of the natural attributes of food were retained. The process showed a good potential for reducing pathogens and spoilage bacteria in the food industry.

The inactivation of microorganisms by HPP is probably the result of a combination of factors, so cell death is due to multiple or accumulated damage inside the cell (Simpson & Gilmour, 1997). Cell death increases with pressure but it does not follow a first-order kinetics; sometimes, there is a tailing off in inactivation (Kalchayanand, Sikes, Dunne, & Ray, 1998). Temperature plays an important role in microbial inactivation for HPP. At optimal growth temperatures, inactivation is less than at higher or lower temperatures of growth because membrane fluidity can be more easily disrupted at temperatures beyond optimal growth (Smelt, 1998). The nature of the suspending media can also affect the resistance pressure of the microorganisms (García-Graells, Masschalck, & Michiels, 1999). In this sense, it is important to experiment with real food matrixes because results obtained in buffers or synthetic media cannot always be extrapolated and applied to real situations. According to Archer (1996), in real food situations, the microbial safety and stability are determined by the effect of food composition both during and after the HPP treatment. The ability of bacteria to survive HPP can be greatly increased when treated in nutritionally rich media, e.g., meat, containing substances like carbohydrates, proteins, and fat (Simpson & Gilmour, 1997).

The commercialisation of food products manufactured under high pressure has produced two different attitudes with regard to regulations both within the European Union and the United States. In the latter, the traditional sanitary regulations are applied. In the European Union, the national regulations for new products have been replaced by a community regulation for “novel food” and ingredients (Regulation EC No 258/97). This legislation establishes a compulsory evaluation and license system for new foods and new processes. HPP foods are classified as “novel foods”. Lately, several decisions have been taken to simplify the regulations. If a “novel food” can be shown to be substantially equivalent to a traditional food already in the market, then it can be treated at a national regulation level without the need to fulfil the “novel food” regulation.

In this study, HPP (600 MPa) at 31 °C during 6 min was assayed in cooked ham, dry-cured ham, and marinated beef loin. The objective was to compare the microbiological evolution between the HPP products and nontreated products during a long chilled storage time (120 days) and thus determine if high-pressure processing is a valid preservation method to reduce the safety risks associated to *Salmonella* and *Listeria monocytogenes*, and

if it avoids or delays effectively the growth of spoilage microorganisms during the chilled storage time evaluated.

2. Materials and methods

2.1. Meat products and process description

Cooked ham, dry cured ham, and marinated beef loin were selected as representative meat products to study.

2.1.1. Cooked ham

Ingredients (in g kg⁻¹): pork ham 884, water 90, salt 17.8, carraghenate 1.0, sodium citrate 1.5, sodium ascorbate 0.5, sodium nitrite 0.12, spices 0.12. Meat was tenderised and injected with a brine containing all the ingredients. Cooking was performed in oven with low-pressure steam, at 67 °C until core temperature reached 65 °C. Standard chemical analysis (in g kg⁻¹): moisture 734, protein 182, carbohydrates 6, fat 50.

2.1.2. Dry cured ham

Ingredients (in g kg⁻¹): pork ham 950, salt 46, dextrose 4, potassium nitrate 0.2. Dry salting with the mixture of ingredients, resting in a cool room (0–4 °C, 85–95% RH) in horizontal layers for salt diffusion, followed by a 40 day post-salting period at 2–6 °C and 70–95% RH. First drying period was carried out in a different room at increasing temperatures of 6–14 °C and at 70–95% RH for 40 days. Ageing–maturation was developed at increasing temperatures of 14–34 °C and at 60–80% RH, until a total processing time of at least 7 months. Standard chemical analysis (in g kg⁻¹): moisture 502, protein 259, carbohydrates 4, fat 160.

2.1.3. Marinated beef loin

Ingredients (in g kg⁻¹): beef loin 943, water 47, salt 10, sodium tripolyphosphate 0.3. Meat was marinated with the brine containing all the ingredients, vacuum-packed, and resting in a cold room for 48–72 h. Standard chemical analysis (in g kg⁻¹): moisture 735, protein 208, carbohydrates 6, fat 35.

2.1.4. Slicing and packaging

The products were sliced and vacuum-packed in 137×250 mm individual packs using a Multivac MP Darfresh equipment (Germany). Five slices (1.2-mm thick each) were packaged in films from Cryovac Europe (Grace, S.A., Sant Boi de Llobregat, 08080 Barcelona, Spain). Bottom film was polystyrene-EVOH based, reference RSC03X60 Darfresh (oxygen permeability 2 cm³/m², 24 h, 1 bar), and upper film was polyethylene-EVOH based, reference TS201 Darfresh Cryovac (oxygen permeability 2 cm³/m², 24 h, 1 bar; water vapour permeability <7 g/24 h, m²). The packaged sliced cooked ham, sliced dry cured ham, and sliced marinated beef

Table 1
Microbial evolution in sliced vacuum-packed cooked ham during storage at 4 °C

Time (days)	Aerobic total count		Psychrotrophs count		Lactic acid bacteria		Yeasts		Enterobacteriaceae	
	NT	HPP	NT	HPP	NT	HPP	NT	HPP	NT	HPP
0	2.45±0.54	2.45±0.54	2.58±0.17	2.58±0.17	<2	<2	<1	<1	<1	<1
After HPP	NA	<2	NA	<2	NA	<2	NA	<1	NA	<1
30	7.59±0.35	2.10±0.18	8.04±0.41	<2	7.84±0.70	<2	2.43±0.75	<1	1.66±1.22	<1
60	8.76±0.04	2.67±1.17	8.76±0.27	<2	8.71±0.22	2.65±1.14	3.00±1.74	<1	<1	<1
90	8.63±0.21	5.64±0.94	8.63±0.20	3.02±1.78	8.57±0.22	5.66±0.78	2.86±0.77	<1	3.71±2.91	<1
120	8.46±0.41	7.48±0.80	8.65±0.40	6.87±0.90	8.76±0.25	7.62±0.97	3.27±1.21	<1	3.08±3.68	<1

NT: nontreated; HPP: treated at 600 MPa; NA: nonapplicable.

Values are mean of triplicate±standard deviation.

Data are expressed in log CFU g⁻¹.

loin were stored for 24 h at 4 °C before pressure treatment.

2.1.5. High-pressure treatment

The choice of pressurisation parameters in this work was mainly influenced by the availability and cost of HPP industrial systems for solid foods. Present equipment offers a maximum operating pressure of 600 MPa in industrial processes. Calculations based in capital costs estimated in four cycles per hour the right production capacity needed to obtain an acceptable cost of treatment. Because the system also needs some time to load, pressurise, depressurise, and unload, the economically reasonable time of treatment at 600 MPa was estimated in a maximum of 6 min. For such reasons, experimental process parameters were fixed at the maximum industrially acceptable values from present available industrial equipment.

The pressurisation was done in an industrial hydrostatic pressurisation unit QFP 35L-600-1 (Flow Pressure Systems Vasteras AB) capable of operating up to 600 MPa. The pressure level (600 MPa), time (6 min), and initial temperature (16 °C) were set by an automatically controlled device. The time needed to achieve the treatment pressure was 135 s, and the decompression time was 45 s. The process water temperature was 16 °C just before HPP, 31 °C during the holding time at 600 MPa (adiabatic heating), and 17 °C just after HPP.

2.1.6. Storage of the samples

After high-pressure treatment, the pressurised samples (HPP) were stored at 4 °C for up to 120 days together with the nontreated control samples (NT). At selected times: time 0, after HPP, and during chilled storage (30, 60, 90, and 120 days), microbiological analyses were carried out in triplicate (three different packages) for each type of product.

2.1.7. Microbiological analyses

Fifteen to twenty grams of sample were aseptically taken and diluted 10-fold in 0.1% Bacto Peptone (Difco Laboratories, Detroit, MI), 0.85% NaCl (Merck, Darmstadt, Germany), blended for 1 min in the Stomacher. Serial dilutions were made and plated onto appropriate culture media to determine aerobic total count in Plate Count Agar (PCA, Merck) at 30 °C, 72 h; psychrotrophs total count in PCA at 8 °C, 7 days; lactic acid bacteria in Lactobacilli MRS Agar (Difco Laboratories) double-layered plates at 30 °C, 72 h in anaerobiosis; Enterobacteriaceae in Violet Red Bile Glucose Agar (VRBG, Merck) double-layered plates at 30 °C, 24 h; yeasts and fungi in Sabouraud Dextrose 2% Agar (SDA, Merck) at 25 °C, 5 days; *Escherichia coli* in Coli ID (bioMérieux, Marcy l'Etoile, France) double-layered plates at 37 °C 48 h. Typical colonies were confirmed by API 20E (bioMérieux); *Staphylococcus aureus* in Baird-Parker (Difco Laboratories) spread plates at 37 °C, 48 h. Typical colonies were confirmed by latex agglutination test

Table 2
Microbial evolution in sliced vacuum-packed dry cured ham during storage at 4 °C

Time (days)	Aerobic total count		Psychrotrophs count		Lactic acid bacteria		Yeasts	
	NT	HPP	NT	HPP	NT	HPP	NT	HPP
0	4.79±0.18	4.79±0.18	2.75±0.62	2.75±0.62	3.33±0.63	3.33±0.63	2.53±0.45	2.53±0.45
After HPP	NA	2.10±0.18	NA	<2	NA	<2	NA	<1
30	4.72±0.25	3.55±0.96	3.94±0.91	<2	2.48±0.53	2.50±0.88	2.16±0.28	<1
60	5.63±1.16	3.25±0.56	4.03±0.72	<2	2.38±0.66	<2	2.23±0.41	<1
90	5.00±0.49	3.09±0.24	4.16±1.30	2.56±0.49	2.35±0.37	<2	2.28±0.49	<1
120	6.50±0.64	2.96±1.68	5.65±0.16	2.81±1.42	2.49±1.00	<2	2.16±0.28	<1

NT: nontreated; HPP: treated at 600 MPa; NA: nonapplicable.

Values are mean of triplicate±standard deviation.

Data are expressed in log CFU g⁻¹.

Table 3
Microbial evolution in vacuum-packed marinated beef loin during storage at 4 °C

Time (days)	Aerobic total count		Psychrotrophs count		Lactic acid bacteria		Yeasts		Enterobacteriaceae		<i>Escherichia coli</i>	
	NT	HPP	NT	HPP	NT	HPP	NT	HPP	NT	HPP	NT	HPP
0	6.51±0.08	6.51±0.08	6.82±0.08	6.82±0.08	6.27±0.04	6.27±0.04	3.29±0.17	3.29±0.17	3.88±0.28	3.88±0.28	1.18±0.22	1.18±0.22
After HPP	NA	<2	NA	<2	NA	<2	NA	<1	NA	<1	NA	<1
30	8.10±0.26	<2	7.92±0.26	<2	8.73±0.02	<2	3.92±0.43	<1	5.46±0.26	<1	1.07±0.20	<1
60	8.57±0.14	<2	8.39±0.14	<2	8.49±0.31	<2	2.91±0.80	<1	5.12±0.74	<1	0.97±0.03	<1
90	8.47±0.18	<2	8.70±0.18	<2	8.70±0.08	<2	2.50±0.17	<1	5.17±0.25	<1	<1	<1
120	8.32±0.15	<2	8.55±0.15	<2	8.68±0.06	<2	2.86±0.07	<1	5.16±0.37	<1	<1	<1

NT: nontreated; HPP: treated at 600 MPa; NA: nonapplicable.

Values are mean of triplicate±standard deviation.

Data are expressed in log CFU g⁻¹.

(Slidex Staph Plus, bioMérieux). Microbiological counts were expressed as log CFU g⁻¹.

L. monocytogenes was investigated in 25 g by pre-enrichment in *Listeria* enrichment broth (UVM I, Oxoid, Basingstoke, Hampshire, England) at 30 °C 24 h, enrichment in UVM II at 30 °C 24 h., followed by selective isolation in Palcam agar (Merck) at 30 °C 24 h. Suspected colonies were confirmed by PCR (Klein & Juneja, 1997). *Salmonella* spp. and *Campylobacter* spp. were investigated according to ISO 6579:1990 (F) and ISO 10272:1995 (F), in 25 and 10 g, respectively. Typical colonies were confirmed by API (bioMérieux). The results were expressed as absence or presence in 25 or 10 g, respectively.

2.1.8. Sensory analyses

During the storage period, HPP samples were checked for the following attributes: off-odours, gas formation, colour changes, and ropiness.

3. Results

3.1. Cooked ham

Lactic acid bacteria (LAB) constituted the main flora in the untreated samples (NT) during the storage period (Table 1). After pressurisation and up to 60 days, the counts were very low (2.65 ± 1.14) log₁₀ CFU g⁻¹ and 6 log below the LAB count obtained in NT samples (8.71 ± 0.22) log₁₀ CFU g⁻¹. However, at the end of the storage (120 days), the counts were similar between HPP (7.62 ± 0.97) log₁₀ CFU g⁻¹ and NT samples (8.76 ± 0.25) log₁₀ CFU g⁻¹.

Yeasts and Enterobacteriaceae (Table 1) showed growth up to 3 log cycles during the storage period (120 days) in untreated samples, while in all HPP samples the number of survivors was kept below the detection limit (<10 CFU g⁻¹) during the whole storage period.

E. coli and *S. aureus* were below the detection threshold, <10 and <10² CFU g⁻¹, respectively, both in HPP and NT samples. *Campylobacter* spp., *L. monocytogenes*, and *Salmonella* spp. showed absence in all the samples (HPP and NT) and during the whole study.

3.2. Dry cured ham

Sliced, skin vacuum-packed dry cured pork ham samples, treated by high-pressure processing at 600 MPa for 6 min, showed a significant reduction of at least 2 log cycles after treatment for total count bacteria, maintaining the survivors at low levels, around 3 log₁₀ CFU g⁻¹, during the storage period (Table 2). Psychrotrophic bacteria were under the detection limit until 60 days after HPP. The counts in NT samples at the end of storage were 6.50 ± 0.64 and 5.65 ± 0.16 (log₁₀ CFU g⁻¹) for

aerobic total count and psychrotrophs, respectively. Enterobacteriaceae and *E. coli* were below the detection threshold, both in HPP and NT samples during the whole storage. *Campylobacter* spp. and *Salmonella* spp. showed absence in all the samples ($n=30$) whereas *L. monocytogenes* was present (in 25 g) in only one untreated sample, at time 0, but absent in all HPP treated samples during the whole 4 °C storage period investigated (120 days).

3.3. Marinated beef loin

Sliced, skin vacuum-packed marinated beef loin, treated by HPP at 600 MPa for 6 min, showed a very significant reduction of at least 4 log cycles after treatment for aerobic, psychrotrophic, and lactic acid bacteria counts (Table 3). The counts of these groups were higher than 10^6 CFU g⁻¹ at time 0. These values, and also the presence of *Salmonella* and *L. monocytogenes* in 25 g in most untreated samples at time 0, pointed out that slaughterhouse operations, handling, or chilled storage before processing have been inappropriate. In HPP samples, the number of survivors remain unchanged and below the detection limit ($<10^2$ CFU g⁻¹) during the whole storage period investigated (120 days), while untreated samples reached 10^8 CFU g⁻¹ after only 30 days of storage.

High-pressure processing was very effective in reducing the Enterobacteriaceae counts nearly 3 log cycles (Table 3), and in keeping them under the detection limit (<10 CFU g⁻¹) during the whole storage period in all HPP samples. Untreated samples (NT) already showed counts of 5.46 ± 0.26 (log₁₀ CFU g⁻¹) after 30 days.

In all HPP samples, *E. coli* and *S. aureus* were kept below the detection limit (<10 or $<10^2$ CFU g⁻¹), respectively, during the whole storage period. *Campylobacter* spp. recorded absence in 10 g in all HPP and NT samples. Nine out of fifteen NT samples showed presence of *L. monocytogenes* in 25 g. Nine out of fifteen NT samples showed presence of *Salmonella* spp. in 25 g. None of the HPP samples ($n=15$) showed presence of either *L. monocytogenes* or *Salmonella* spp. in 25 g at any time during the whole storage period (120 days) (Table 4).

Table 4
Investigation of *L. monocytogenes* and *Salmonella* spp. in marinated beef loin during storage at 4 °C

Time (days)	<i>L. monocytogenes</i>		<i>Salmonella</i> spp.	
	NT	HPP	NT	HPP
0	2/3	0/3	3/3	0/3
30	2/3	0/3	2/3	0/3
60	3/3	0/3	2/3	0/3
90	1/3	0/3	0/3	0/3
120	1/3	0/3	2/3	0/3

NT: nontreated; HPP: treated at 600 MPa.

Data are expressed as number of positive samples (presence in 25 g)/number of investigated samples.

4. Discussion

The efficacy of high-pressure treatments for the inactivation of vegetative bacteria in foods has been reported previously (Cheftel, 1995; Farkas & Hoover, 2000; Smelt, 1998; Yuste et al., 2001). However, there are limited studies on the evaluation of microbial safety and quality of pressurized products during chilled storage (Carpi et al., 1999; López-Caballero, Carballo, & Jiménez-Colmenero, 1999; Yuste, Pla, Capellas, Ponce, & Mor-Mur, 2000). Often, food composition can have a protective effect during pressurisation, and it is important to evaluate microbial resistance to pressure in foods rather than in traditional buffer solutions.

Marketing of convenience foods is increasing due to consumer demand. Slicing and packaging operations take place after cooking, and cross-contamination at these points is critical regarding the shelf life and safety of the products. In this study, microorganisms were present at very low levels in freshly sliced packaged cooked ham, suggesting that the hygienic conditions observed in the enterprise were correct. Because of the high water activity of cooked ham (0.978), lactic acid bacteria, mainly coming from cross-contamination during slicing and packaging, grew quickly to 10^8 CFU g⁻¹ in all the untreated samples in 30 days, whereas the pressurized samples showed a significant delay in the growth of spoilage associated microorganisms compared to untreated samples, contributing to the maintenance of the sensorial freshness for at least 60 days after treatment. Even after 90 days, the counts did not reach the level defined as spoilage level (10^7 CFU g⁻¹). The HPP process helped to prevent off-odours, ropiness, and colour changes. Carpi et al. (1999) reported an extended shelf life of sliced cooked ham treated at 600 MPa for 5 min up to 75 days stored at 4 °C. The results achieved by López-Caballero et al. (1999) with the same type of product but treated at lower pressures (200 MPa or 400 MPa) did not reach the same degree of inactivation and the maximum extent of shelf life obtained at 3 °C was up to 21 days, for sliced and pressurized cooked ham (400 MPa for 20 min). These results agree with the generally accepted fact that the degree of inactivation is directly related to the level of pressure applied.

High-pressure processing, in the conditions used in this assay, was an effective process to avoid the growth of yeasts and Enterobacteriaceae with a potential to produce off-flavours and gas. It is generally felt that for microorganisms, the primary site of pressure damage is the cell membrane. Pressures of 200–400 atm can disrupt the stressed cell wall, and this may be a primary factor for yeasts (Hoover, Metrick, Papineau, Farkas, & Knorr, 1989). Eucaryotic microorganisms are generally more sensitive to pressure than procaryotic microorganisms. In general, Gram-negative bacteria are more sensitive to pressure than Gram-positive bacteria (Carlez, Rosec, Richard & Cheftel, 1994; Shige-hisa, Ohmori, Saito, Taji, & Hayashi, 1991). In fact, for all

the products studied, no further recovery of yeasts or Enterobacteria including *E. coli* was recorded in HPP samples during the whole storage period.

Dry cured ham, is a raw, bone-in, dried, nonfermented meat product. Because of the low water activity (0.890) and high salt content (4.60%) of this product, spoilage microorganisms are mainly Gram-positive cocci and yeasts. They are present at the surface of the whole ham, and they reach the sliced product during final boning, slicing, and packaging operations. In general, low water activity protects cells against pressure (Cheftel & Culioli, 1997), but microorganisms injured by pressure are generally more sensitive to low water activity. After high-pressure treatment, the survivors were maintained at low levels during the storage period, contributing to the preservation of the sensorial freshness for 120 days. The marked pressure sensitivity of psychrotrophs observed in this product as well as in cooked ham, compared with the aerobic total count (mesophiles), has also been reported by other authors (Yuste et al., 2000). It seems that psychrotrophs lose the ability to grow at refrigeration temperature as a consequence of heat or pressure processing.

Marinated beef loin is a raw meat product with high water activity (0.985), low level of salt (1%), without nitrite, and with a mixed flora of spoilage microorganisms and pathogens from the slaughterhouse cutting and trimming operations. High-pressure treatment was very effective in reducing all the microbial groups investigated, probably because of the high water activity of this product; no further recovery of survivors was recorded during the storage. Carlez et al. (1994) reported the total absence of lactic acid bacterial growth, and a delay of 13–15 days for aerobic total count in minced beef treated at 450 MPa and storage at 3 °C.

The results obtained in this study—an extensive cell inactivation ratio (>4 log cycles) without reactivation capacity, starting with an important initial contamination level of 6 log CFU g⁻¹—agree with the fact that the higher the pressure, the higher the inactivation obtained. HPP is a powerful tool to control risks associated with *Salmonella* spp. and *L. monocytogenes* in raw or marinated meats as shown from the data obtained in this study. Most of the untreated samples (NT) showed the presence of one or both pathogens in 25 g, whereas all pressurized samples (HPP) showed the absence of these pathogens in 25 g. However, colour modifications (greyish colour) were observed in pressurized samples. Colour modifications in minced beef muscle treated at pressures above 350 MPa was also reported by Carlez, Rosec, Richard, and Cheftel (1993).

Cheftel and Culioli (1997) suggested prepackaged sliced cooked ham or salami as good candidates for high-pressure “pasteurisation” because pink or red colour resist high pressure. In this study, no visual differences were observed between NT and HPP samples of cooked ham and dry cured ham. Meat discoloration in raw meat from pressure processing may result from an oxidation of ferrous

myoglobin to ferric myoglobin at pressures equal to or greater than 400 MPa (Carlez, Veciana-Nogues, & Cheftel, 1995). As suggested by Farkas, Hajós, Kaffka, Mészáros, and Szerdahelyi (2001), some research needs to be done to determine if high-pressure-induced discoloration of muscle pigments could be prevented by nitrite addition.

Although the initial capital expenditure is still costly, pressure treatments consume less energy than thermal processing, which suggests that the products would be commercially competitive. It is necessary to keep in mind that the degree of cellular disruption of the food greatly affects its sensory properties.

High-pressure pasteurized products such as guacamole and oysters are currently being marketed in the United States, and jams, jellies, fish, sliced ham, salad dressing, juices, and yogurt are being marketed in Japan and Europe. In Spain, a meat company is using an ACB Pressure System machine for the pressure treatment (at 400 MPa) of sliced cooked and dry cured ham. These products have been commercialised in Spain since 1998, and they claim the same level of freshness throughout the 60 days as for freshly sliced ham. In the United States, a meat company is using Flow International equipment for general meat decontamination and for the elimination of *L. monocytogenes*. In this country, the authorities maintain a zero tolerance for *L. monocytogenes*.

In conclusion, high-pressure processing at 600 MPa for 6 min at 31 °C is an efficient method for delaying the growth of spoilage microorganisms in all the sliced vacuum-packed meat products investigated, and is also an efficient method for reducing the safety risks associated with *Salmonella* and *L. monocytogenes* in sliced marinated beef loin. The composition of the food product is perhaps one of the key factors influencing the preservative effect of HPP.

Acknowledgements

This work was supported by projects ALI1998-0709 and FIT060000200066PROFIT (Esteban Espuña, S.A.).

References

- Archer, D. L. (1996). Preservation microbiology and safety: Evidence that stress enhances virulence and triggers adaptive mutations. *Trends in Food Science and Technology*, 7, 91–95.
- Carlez, A., Rosec, J. P., Richard, N., & Cheftel, J. C. V. (1993). High pressure inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens*, and *Listeria innocua* in inoculated minced beef muscle. *Lebensmittel-Wissenschaft + Technologie*, 26, 357–363.
- Carlez, A., Rosec, J. P., Richard, N., & Cheftel, J. C. V. (1994). Bacterial growth during chilled storage of pressure-treated minced meat. *Lebensmittel-Wissenschaft + Technologie*, 27, 48–54.
- Carlez, A., Veciana-Nogues, T., & Cheftel, J. C. (1995). Changes in colour and myoglobin of minced meat due to high pressure processing. *Lebensmittel-Wissenschaft + Technologie*, 28, 528–538.

- Carpi, G., Squarcina, N., Gola, S., Rovere, P., Pedrielli, R., & Bergamaschi, M. (1999). Application of high pressure treatment to prolong the refrigerated shelf-life of sliced cooked ham. *Industria Conserve*, 74, 327–339.
- Cheftel, J. C. (1995). Review: High-pressure, microbial inactivation and food preservation. *Food Science and Technology International*, 1(2/3), 75–90.
- Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: A review. *Meat Science*, 46(3), 211–236.
- Farkas, D., & Hoover, D. (2000). High pressure processing pp. 47–64 in IFT. 2000. Special supplement: Kinetics of microbial inactivation for alternative processing technologies. Inst. of Food Technologists. *Journal of Food Science*, 65(8), S1–S108.
- Farkas, J., Hajós, Gy., Kafka, K., Mészáros, L., & Szerdahelyi, E. (2001). Studies on high pressure induced physicochemical changes in minced meat. Proceedings European Conference on Advanced Technology for Safe and High Quality Foods. December 2001, Berlin, Germany.
- García-Graells, C., Masschalck, B., & Michiels, C. W. (1999). Inactivation of *Escherichia coli* in milk by high-hydrostatic-pressure treatment in combination with antimicrobial peptides. *Journal of Food Protection*, 62, 1248–1254.
- Hoover, D. G., Metrick, C., Papineau, A. M., Farkas, D. F., & Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. *Food Technology*, 43, 99–107.
- ISO 6579 (1990) (F). Microbiologie. Directives générales concernant les méthodes de recherche des *Salmonella*. International Organization for Standardization. Geneva, Switzerland.
- ISO 10272 (1995) (F). Méthode horizontale pour la recherche des *Campylobacter* thermotolérants. International Organization for Standardization. Geneva, Switzerland.
- Kalchayanand, N., Sikes, A., Dunne, C. P., & Ray, B. (1998). Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. *Food Microbiology*, 15, 207–214.
- Klein, P. G., & Juneja, V. K. (1997). Sensitive detection of viable *Listeria monocytogenes* by reverse transcription-PCR. *Applied and Environmental Microbiology*, 63(11), 4441–4448.
- López-Caballero, M. E., Carballo, J., & Jiménez-Colmenero, F. (1999). Microbiological changes in pressurized, prepackaged sliced cooked ham. *Journal of Food Protection*, 62(12), 1411–1415.
- Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal L 043, 14/02/1997 P. 0001-0006.
- Shigehisa, T., Ohmori, T., Saito, A., Taji, S., & Hayashi, R. (1991). Effects of high hydrostatic pressure on characteristics of pork slurries and inactivation of microorganisms associated with meat and meat products. *International Journal of Food Microbiology*, 12, 207–216.
- Simpson, R. K., & Gilmour, A. (1997). The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems. *Journal of Applied Microbiology*, 83, 181–188.
- Smelt, J. P. P. M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology*, 9, 152–158.
- Yuste, J., Pla, R., Capellas, M., Ponce, E., & Mor-Mur, M. (2000). High pressure processing applied to cooked sausages: Bacterial population during chilled storage. *Journal of Food Protection*, 63(8), 1093–1099.
- Yuste, J., Pla, R., Capellas, M., Sendra, E., Beltran, E., & Mor-Mur, M. (2001). Oscillatory high pressure processing applied to mechanically recovered poultry meat for bacterial inactivation. *Journal of Food Science*, 66(3), 482–484.