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Authors: Anne Vercammen[*1], Kristof G. A. Vanoirbeek[1], Ine Lurquin[1], Liselot Steen[3], Olivier Goemaere[3], Slawomir Szczepaniak[3], Hubert Paelinck[3], Marc E.G. Hendrickx[2] and Chris W. Michiels[1*]

Affiliations

[1]Laboratory of Food Microbiology, [2]Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LFoRCe), Department of Microbial and Molecular Systems (M^2S), Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, B-3001 Leuven, Belgium.

[3]Research group for Technology and Quality of Animal Products and Leuven Food Science and Nutrition Research Centre (LFoRCe), KaHo Sint-Lieven - Technologiecampus Gent, Gebroeders Desmetstraat 1, 9000 Gent, Belgium

[*]corresponding author

Phone: +32-16-321578

Fax:+32-16-321960

Email:Chris.michiels@biw.kuleuven.be

Abstract

The inactivation of different spoilage organisms and surrogate pathogens in a cooked ham model product by high pressure (HP) treatment (100-700 MPa, 5-40 °C, 10 min) was investigated. A 5 log reduction could be achieved at ≥ 600 MPa at ≥ 25 °C. Subsequently, the shelf-life of packaged sliced product was studied during storage (7 °C) after treatment at 600 MPa (10 °C, 10 min) in combination with caprylic acid and Purasal®. Without HP treatment, a plate count of 6 log CFU/g was reached after 40 days, both in presence and absence of antimicrobials. HP treatment delayed this initiation of spoilage to 59 days in absence of antimicrobials. However, microbial growth was completely suppressed during at least 84 days in the HP treated products containing caprylic acid or Purasal®. HP treatment increased drip loss but had no or little effect on colour and sensorial evaluation by a taste panel. However, the antimicrobials had a negative influence on the flavour and aroma at the concentrations used.

Keywords: high hydrostatic pressure, cooked ham model product, shelf-life, quality, microorganisms, antimicrobials

Industrial relevance

With a steadily increasing number of commercial applications being introduced on the market, HP pasteurization is growing out of its infancy. To further support this development, there is a need of integrated studies that translate fundamental scientific findings from simplified laboratory model systems to the complexity and scale of real food products. In this work, we

determined HP processing conditions to control spoilage and pathogenic bacteria in a cooked ham model product, and subsequently conducted a large pilot scale experiment comprising a total of 432 individual packages of sliced cooked ham product, in which the microbiological, physicochemical and sensorial quality was evaluated during refrigerated storage after HP treatment. In addition, the usefulness of the natural preservatives caprylic acid and lactate-diacetate as an additional hurdle was also studied. This study is one of the most comprehensive available in the literature to document the shelf-life extension that can be achieved with HP treatment of cooked ham.

1. Introduction

The shelf-life of cooked and sliced meat products, like cooked ham, is limited mainly because of microbiological safety and spoilage issues. This is because manipulations like slicing and packaging unavoidably reintroduce bacterial contaminants after the cooking process, and because the product has a near-neutral pH (around 6) and water activity of higher than 0.945 (Hu, Zhou, Xu, Li, & Han, 2009). As such, the shelf-life depends on –besides intrinsic product parameters- good hygienic practices, control of the distribution and storage temperature and vacuum or modified atmosphere packaging. The commercial shelf-life of vacuum packaged cooked ham can be typically up to 30 days at 1-8 °C (Hu et al., 2009). Spoilage of packaged sliced ham is mostly accompanied by souring, slimy meat juice exudates and swelling of the pack due to gas production (Samelis, Kakouri, Georgiadou, & Metaxopoulos, 1998), and is usually caused by Lactic Acid Bacteria (LAB). The main genera implicated are *Lactobacillus* and *Leuconostoc*, with *Lb. sake* and *Lb. curvatus* being isolated commonly (Holzapfel, & Gerber,

1986; Dykes, Britz, & von Holy, 1994; Dykes, Cloete, & von Holy, 1995). With respect to safety, contamination with pathogens like *Escherichia coli* O157:H7 or *Salmonella* is a concern, but the most important hazard is without any doubt *Listeria monocytogenes*, because of its widespread environmental distribution and ability to grow in refrigerated products (Beumer, te Giffel, de Boer, & Rombouts, 1996; Ryser, & Marth, 1999; Uyttendaele De Troy, & Debevere, 1999; Talon et al., 2007).

Several studies have addressed the use of high hydrostatic pressure (HP) treatment as an in-package final process step to eliminate post-cooking contamination of ready-to-eat (RTE) meat products and thus increase their safety and shelf-life, and these have been recently reviewed (Garriga, & Aymerich, 2009). In general, these studies indicate that 600 MPa treatment (5 – 10 min, 15 – 30 °C) is needed to sufficiently reduce the levels of the pathogens *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* and to prevent their outgrowth during subsequent refrigerated storage, particularly in case of a simulated cold chain break (Aymerich, Jofré, Garriga, & Hugas, 2005; Marcos, Jofré, Aymerich, Monfort, & Garriga, 2008; Jofré, Aymerich, Grèbol, & Garriga, 2009). Characteristic spoilage microbiota like *Enterobacteriaceae, Brochothrix thermosphacta* and LAB were also reduced by HP treatment, but only the latter were consistently found to resume growth up to high numbers during subsequent cold storage even for pressure treatments at 600 MPa and storage at 2 °C, while the former two did not reappear when the treatment pressure was 400 MPa or more (López-Caballero, Carballo, & Jiménez-Colmenero, 1999; Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004; Jofré et al., 2009). Based on this observation Jofré et al. (2009) suggested that HP treatment of cooked ham should be complemented with the use of additional hurdles to further reduce outgrowth of LAB. The color, pH-value and texture of cooked meat products are not affected by HP treatment, but an increase

in drip loss was reported in one study (Cheftel, & Culioli, 1997; López-Caballero et al., 1999; Hayman, Baxter, O'Riordan, & Stewart, 2004; Pietrzak, Fonberg-Broczek, Mucka, & Windyga, 2007).

To further improve the efficacy of HP treatment or to achieve similar efficacy at lower pressures, some authors have explored hurdle technology by combining HP with natural antimicrobials like bacteriocins, lactate and a lactate-diacetate mixture. This approach allowed higher pathogen reduction by HP and longer suppression of pathogen outgrowth after HP treatment. The effect of these combination treatments on the microbiological shelf-life was not addressed in these studies (Garriga, Aymerich, Costa, Monfort, & Hugas, 2002; Aymerich et al., 2005; Jofré, Garriga, & Aymerich, 2008; Marcos et al., 2008).

Although there are a considerable amount of studies on HP treatment of sliced cooked ham in the literature, they are often difficult to compare, and there is a lack of comprehensive studies that cover a wide range of experimental conditions and output variables. Table 1 summarizes the experimental conditions and output variables from the studies that we have found in the literature. In our own work (last entry in Table 1), HP inactivation of both relevant spoilage organisms and (surrogate) pathogens is determined, and this is done over a much wider range of process conditions (100-700 MPa; 5, 25, 40°C) than in any other study. The approach was to first optimize pressure treatment conditions towards sufficient (5- 6 log) inactivation of specific spoilage bacteria and surrogate pathogens including pressure resistant strains at lab scale, and subsequently conduct a pilot scale trial using optimized process conditions. Since HP alone could not achieve the desired level of inactivation for all strains at the lowest process temperature, two natural antimicrobials (caprylic acid and a lactate-diacetate mixture) were added to the ham formulation in the pilot scale trial. No other study has used caprylic acid (or any other short chain

fatty acid), and lactate-diacetate has only been studied with respect to *L. monocytogenes* control, not for overall microbiological control. Further, while previous studies were always conducted with vacuum packed products, we used modified atmosphere packed (MAP) products, thereby introducing an additional hurdle. Finally, compared to previous studies, we analysed the most extensive set of output variables, covering not only a wide range of microbiological parameters (aerobic mesophilic count, lactic acid bacteria, anaerobic mesophilic bacteria, sulfite reducing clostridia, *Brochothrix* spp. and *Enterobacteriaceae*), but also quality parameters, including colour, pH, water binding and sensory evaluation.

2. Materials and methods

2.1. Cooked ham model product

Preselected ham meat (*M. semimembranosus*, *M. semitendinosus* and *M. biceps femoris*, PQM ~9 mS/cm, T= 4 °C), cut to an average size of 30 mm, was used to prepare the cooked ham model product. The meat was tumbled (120 min, 10 rpm) at 3°C with a brine solution (20 g brine/100 g preselected ham meat) containing various ingredients: salt, sodium nitrite, sodium ascorbate, glucose, phosphate (Deraphos, Dera Food Technology, Bornem, Belgium) and, in some cases, caprylic acid or Purasal® OptiForm PD Plus. The latter is a commercial mixture containing 72.8% potassium lactate and 5.2% sodium diacetate as active ingredients (Purac Biochem, Gorinchem, The Netherlands). The final concentrations of these ingredients in the finished product are 18 g/kg salt, 0.120 g/kg sodium nitrite, 0.5 g/kg sodium ascorbate, 5 g/kg glucose, 3 g/kg phosphate and, in some cases, 1.5 g/kg caprylic acid or 25 g/kg Purasal® OptiForm PD Plus. The maximum amount of sodium nitrite that may be added during manufacturing of meat products is 150 mg sodium nitrite/kg (Directive 2006/52/EC). After tumbling, the product was packaged in narrow (d= 30 mm, l= 20 cm) or in wide (d = 110 mm, l

= 20 cm**)** synthetic cylindrical casings (Dera Food Technology, Bornem, Belgium) for lab scale and pilot scale HP experiments respectively. The product was then cooked under saturated vapour conditions in a steam oven (1600 EL, Kerres, Backnang, Germany) at 72 °C, until the core temperature of 68 °C was reached. After cooking the product was immediately cooled in an ice bath until the core temperature was 4 °C. Then, the product for the lab scale experiments was stored at -20 °C prior to HP treatments, and the product for the pilot scale experiment was sliced and MAP packaged (70% N_2 and 30% CO_2) in portions of approximately 100 g in sterile polyethylene bags (180mmx300mmx70µm, Grade Packaging Ltd., Leichestershire, England) and stored at 4 °C for up to 1 day prior to the HP experiment.

2.2. Bacterial strains and culture conditions

Carnobacterium divergens M6, *Leuconostoc carnosum* M3 and *Brochothrix thermosphacta* M1 are cooked ham isolates from the culture collection of KaHo Sint-Lieven (Ghent, Belgium). *Lactobacillus sakei* DSM6333 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and was originally isolated from vacuum-packaged pork meat. *Listeria innocua* CIP80.11 and CIP80.12 were obtained from the culture collection of the Institute Pasteur (Paris, France) and were originally isolated from respectively bovine brain and human feaces. The non-toxigenic *E. coli* O157:H7 strain ATCC43888 was obtained from the American Type Culture Collection (Manassas, VA, USA). *E. coli* LMM1010 is a HP resistant derivative of *E. coli* MG1655 (Hauben, 1998), and *E. coli* LMM1080 is a HP resistant derivative of ATCC43888 selected in our laboratory (unpublished results).

Bacterial cultures were grown at 30 °C for *L. innocua* and *Lb. sakei,* at 25 °C for *C. divergens*, *Lc. carnosum* and *B. thermosphacta* and at 37 °C for *E. coli.* Culture media used were Brain

Heart Infusion (BHI, Oxoid, Basingstoke, United Kingdom) for *C. divergens*, *Lc. carnosum*, *B. thermosphacta* and *L. innocua*, de Man Rogosa Sharp (MRS, Oxoid) for *Lb. sakei* and Luria Bertani containing 10 g/1 tryptone (Lab M, Amersham, England), 5 g/l yeast extract (Oxoid) and 5 g/1 NaCl for *E. coli*. Cultures were inoculated from a single colony on an agar plate and grown well-aerated to stationary phase for 21 h. Cells were harvested by centrifugation (4000x*g*, 5 min), washed two times and resuspended in sterile deionised water to a cell density of approximately 10^9 CFU/ml.

2.3. Lab scale HP treatments

To study HP inactivation of specific spoilage bacteria and (surrogate) pathogens, HP pressure treatments (100 – 700 MPa, 10 min holding time) were carried out in a multivessel high pressure equipment (8x8 ml, HPIU-10000, 95/1994, Resato, Roden, The Nederlands) that was temperature controlled by means of a water jacket. Initial temperatures in the vessel were 5, 25 or 40 °C. The temperature increase in the vessels during pressurization was 5-8 °C depending on the pressure applied, as described previously (Van Opstal, Vanmuysen, Wuytack, Masschalck, & Michiels, 2005). Cooked ham model product was sliced aseptically into portions of 1±0.1 g and transferred to small sterile polyethylene pouches. Subsequently, 0.1 ml of a bacterial suspension prepared as described above was added to obtain about 10^8 CFU/g meat product. Pouches were heat sealed and held on ice water for one hour before HP treatment.

2.4. Pilot scale HP treatment

For studying the effect of HP treatment on product quality during subsequent storage, a pilot scale HP treatment was conducted with 100 g packages of MAP packed sliced ham prepared as described above. HP treatment was at 600 MPa with a 10 min holding time, and was performed

in a Wave 6000/55 discontinuous hydrostatic pressurization unit (NC Hyperbaric, Burgos, Spain). The water of the pressurizing system was 10 °C at the beginning of the experiment, and maximally reached 16-18°C during pressurization at 600 MPa. 72 packages each of cooked ham without additives, with 2.5% Purasal® and with 0.15% caprylic acid were HP treated in a single run, and stored for 84 days at 7 °C, along with an equal number of non-pressurized controls. At 12 specific intervals during this period, three replicate packages were taken for microbiological analysis, and three for analysis of the other quality parameters.

2.5. Microbiological analysis

The survival of bacteria inoculated on the 1 g ham samples after lab scale HP treatment was determined by plate count. Samples were homogenized in 9 ml sterile potassium phosphate buffer (10 mM, pH 7.0) (PP buffer) for 30 seconds and serial dilutions in PP buffer were spread-plated on tryptone soy agar (TSA, Oxoid) with 0.6% yeast extract (Oxoid) for *C. divergens*, *Lc. carnosum*, *B. thermosphacta* and *L. innocua*, on MRS for *Lb. sakei* and on LB for *E. coli*. Incubation temperatures were the same as used for growing the inocula. The lower detection limit for these experiments is 2 log CFU/g meat. Data are expressed as log CFU/g indicating the number of cells surviving the pressure treatment.

The packages from the pilot scale shelf-life experiment were aseptically opened and the entire content was homogenized in 200 ml of PP buffer during 30 seconds in a stomacher (Seward 400 circulator, London, England). Serial dilutions in PP buffer were pour-plated for determination of aerobic mesophilic bacteria on Plate Count agar (PCA, Oxoid, incubation at 30 °C for 2 d), for LAB on MRS agar (Oxoid, oxic incubation at 30 °C for 5 d), for anaerobic mesophilic bacteria on Reinforced Clostridium Agar (RCA, Oxoid, anoxic incubation at 37 °C for 2 d), for sulfite

reducing clostridia on Differential Reinforced Clostridial medium (DRCM, Oxoid, anoxic incubation at 37 °C for 7 d), for *Brochothrix* spp. on Streptomycin Thallium Acetate Agar (STAA, Oxoid, incubation at 22 °C for 2 d) and for *Enterobacteriaceae* on Violet Red Bile agar with Glucose (VRBG, Oxoid, incubation at 37 °C for 1 d). For counting of aerobic and anaerobic spores, samples were heated for 30 min at 70 °C and plated on PCA and RCA respectively. The lower detection limit for these determinations was 0.5 log CFU/g meat. Data are expressed as log CFU/g indicating the amount of cells inside a package.

2.6. Determination of water binding capacity

The water binding capacity was measured at predetermined time intervals after HP treatment by means of a Kg-test and measurement of the amount of syneresis.

Kg-test

Cooked ham model product was homogenized with a blender (2096 Homogeniser, FOSS N.V., Brussels, Belgium) and 0.3 g of the homogenized sample was placed on a 45 mm filter paper between two glass plates. A weight of 1 kg was placed on top of these plates during 5 minutes. The ratio of the surface over which the meat sample had been squeezed to the surface over which the squeezed out moisture had spread was calculated as a measure of the water binding ability of the meat sample.

Syneresis

Immediately after HP treatment, sliced samples were stored at a vertical angle of 45 ° at 7 °C. Syneresis was expressed as the % fluid loss as follows:

$$S= [((W_{total}-W_{package})-W_{Sample})/(W_{total}-W_{package})]*100$$

with S: syneresis

W_{total} : weight of package with meat and released fluid

$W_{package}$: average weight of 50 empty packages

W_{sample} : weight of meat without released fluid

2.7. Colour measurement

A portable Minolta Chromameter (CR-300, Osaka, Japan) was used to measure the colour of the meat samples after homogenisation with a blender (2096 Homogeniser, FOSS N.V., Brussels Belgium). Colour coordinates were determined according to the CIE-lab system and the results were expressed as lightness (L^*), redness (a^*) and yellowness (b^*).

2.8. Determination of meat pH-value

The pH-value of the homogenized meat was measured using a pH meter (Knick Portamess, type 911, Elscolab N.V., Kruibeke, Belgium).

2.9. Quantitative descriptive analysis (QDA)

The sensorial properties of the HP treated and untreated cooked ham model product were assessed after two weeks of storage at 7 °C by means of QDA using a trained taste panel (12 persons) according to the method of Meilgaard, Civille, and Carr (1999). Parameters that were scored included flavour intensity, aroma (odor) intensity, colour, juiciness and tenderness.

2.10. Reproducibility of results

For the lab scale experiments HP treatments were conducted in triplo and data are expressed as mean value ± standard deviation on the treatment. For the microbiological experiments on pilot scale data are expressed as mean value ± standard deviation for three replicate packages at each time point. For the other quality parameters the data were analyzed by one-way analysis of variance using SPSS 16.0 (SPSS inc., Chicago, USA) and differences among the means were compared using Tukey's-t test. A significance level of 0.05 was chosen.

3. Results

3.1. HP inactivation of specific spoilage bacteria in cooked ham model product: lab scale study

Cooked ham model product was inoculated with different spoilage organisms and HP treated at 100 to 700 MPa for 10 min at an intial temperature of 5, 25 or 40 °C. The numbers of surviving bacteria after these treatments are shown in Figure 1.

The background level of aerobic mesophilic bacteria in the cooked ham model product was less than 2 log CFU/g, and thus did not disturb measurement of the surviving inoculated bacteria. In general, little or no inactivation of any of the four bacteria occurred up to 300 MPa, except at 40 °C, where inactivation levels were varied, with 0.4 log CFU/g for *Lb. sakei* DSM6333, 1.3 log CFU/g for *Lc. carnosum* M3, 2.5 log CFU/g for *C. divergens* M6 and 3.1 log CFU/g for *B. thermosphacta* M1.

Treatment at 400 MPa caused inactivation for all bacteria, and the levels were at least 2 – 3 log higher than at 300 MPa. At 500 MPa and higher, the number of survivors was mostly below the detection limit (2 log CFU/g). The most notable exceptions are the survival of 3.6 log CFU/g of *C. divergens* at 500 MPa (25 °C), and of 2.1 log CFU/g of *Lb. sakei* at 600 MPa (5 °C) and 2.1

log CFU/g of *B. thermosphacta* at 700 MPa (5 °C). Although in general the differences in HP sensitivity between the four tested bacteria were small.

3.2. HP inactivation of surrogate pathogens in cooked ham model product: lab scale study

A similar experiment as above was conducted to assess the HP inactivation of three *E. coli* and 2 *L. innocua* strains as surrogate pathogens (Figure 2). A simple visual comparison of the general appearance of Figures 1 and 2 allows to detect some parallels but also differences in the general HP inactivation behaviour of the spoilage bacteria and the surrogate pathogens. For the spoilage bacteria, the threshold pressure needed to cause inactivation of the surrogate pathogens was around 300 – 400 MPa, and inactivation generally increased with temperature, particularly from 25 to 40 °C. On the other hand, the surrogate pathogens in general tend to show higher survival in the 500 – 700 MPa pressure range, indicating a lower pressure dependence of the inactivation compared to the spoilage bacteria. As an illustration of this behaviour, four out of the five surrogate pathogens were not completely inactivated in the 700 MPa treatment at 5 °C and in the 600 MPa treatment at 25 °C, although the number of survivors was low (< 3 log CFU/g). In fact, treatment at 600 or 700 MPa reduced all surrogate pathogens by at least 4.8 log at all temperatures, except for the pressure resistant *E. coli* LMM1010 at 5 °C, where only about 2 log reduction was obtained at both 600 and 700 MPa. There appeared to be no consistent difference in HP resistance between the *E. coli* and *L. innocua* strains in this experiment.

3.3. Shelf-life of HP treated cooked ham model product: pilot scale study

Microbiological aspects

Based on the results of the lab scale experiments, and considering that most industrial HP machines are not temperature controlled and usually operate in refrigerated rooms (10 °C in this

study), we decided to use the maximal possible pressure of 600 MPa for the pilot scale experiment. This pressure level would be needed to achieve a 5 - 6 log reduction of vegetative pathogens and spoilage bacteria, except for extremely HP resistant strains if these would be present in the product. Since this is not a very strong reduction, caprylic acid (0.15%) and Purasal® (2.5%) were included in the pilot scale experiment as additional hurdles that could be helpful to extend the shelf-life. Preliminary experiments at lab scale had shown that the HP inactivation of *C. divergens* M6, *E. coli* LMM1010 and *L. innocua* CIP80.12 inoculated on cooked ham was not significantly affected by these antimicrobials (data not shown), but this does not exclude a possible effect on outgrowth of surviving bacteria during subsequent storage. The results of the pilot scale experiment are shown in Table 2.

The initial aerobic mesophilic count of the unpressurized cooked ham model product with or without caprylic acid or Purasal® was approximately 1 log CFU/g. In the absence of additives, an increase in the aerobic mesophilic count and LAB was first seen after 15 d in at least some individual packages of this unpressurized product, and after respectively 40 d and 45 d, the mean value for these parameters surpassed 6 log CFU/g, a value that can be considered as spoilage threshold. The antimicrobial additives delayed bacterial development. The first packages showing an increase of aerobic mesophilic count and LAB were observed respectively after 26 d and 33 d for caprylic acid, and after 26 d and 15 d for Purasal®. From that time onwards, the average counts generally increased, although the trends were not always smooth, probably because of a large pack-to-pack variability and the fact that for practical reasons only three replicate packages were included for each time point. The threshold of 6 log CFU/g was occasionally exceeded for both parameters after 33 to 40 d.

HP treatment also delayed bacterial development. In the absence of additives, an increase of the aerobic mesophilic count was observed in some packages from 40 d onwards and some packages exceeded the threshold after 59 d. Interestingly, the combination of HP treatment and caprylic acid or Purasal® completely stabilized the product for the entire storage period of 84 d, since there was not a single package that showed a significant increase in mesophilic aerobic count or LAB (highest recorded value: 1.3 log CFU/g). Several other microbiological parameters were monitored in this pilot scale experiment, but these were not very informative since the initial counts were always below 1.0 log CFU/ml, and because no important increases were recorded during storage of the HP treated samples. More specifically, the counts of anaerobic mesophilic counts, sulphite reducing clostridia, *Brochothrix* spp. or *Enterobacteriaceae* did not increase in any of the samples, and those of the aerobic and anaerobic spores never exceeded 2.4 log CFU/g throughout the experiment (data not shown).

Quality characteristics

Further experiments were conducted to determine the effect of HP on the water binding capacity, the colour and the pH-value of the cooked ham model product during cold storage. In general, a statistically significant increase of syneresis was observed from approximately 2% for the unpressurized control to 4-5% immediately after HP treatment, but no significant changes were observed with the kg-test (data not shown). Overall, syneresis remained relatively stable over the 10 week storage period (Figure 3).

There was no visual difference in colour between HP treated or untreated products, and between products with or without antimicrobial additives over the entire duration of the experiment, but analytical measurement revealed a slight but significant increase in the L^* value (lightness) when

caprylic acid or Purasal® was present. During storage the ham turned somewhat greyish, and showed a significant increase in yellowness (b^* value) and a significant decrease in redness (a^* value) (data not shown).

In general, HP had no influence on the pH-value of the meat and no significant changes of pH-value were observed during refrigerated storage, irrespective of HP treatment or presence of additives. The initial product pH-value was 6.0 in the absence and 5.85 in the presence of additives, and, except for a slight transient decrease of about 0.15 pH units in all samples after 1 week, remained constant over the entire storage duration.

After 14 d, a QDA test with an experienced taste panel was conducted. No statistically significant differences between the HP treated and the untreated samples were detected for any of the descriptors. However, the presence of caprylic acid and Purasal® had a negative impact on the flavour and the aroma.

4. Discussion

The general objective of this work was to investigate the shelf-life extension of cooked ham type products by HP treatment in combination with MAP and some natural preservatives. As a first step, we conducted lab scale experiments using a simplified setup (vacuum-packaged product without preservatives) to determine the approximate process conditions that would sufficiently reduce the typical spoilage bacteria and the most relevant pathogens in these products. Using four different specific spoilage lactic acid bacteria, process conditions to achieve a 5 to 6 log reduction were delineated. Since the initial inoculum density in the cooked ham model product varied from approximately 7 log CFU/g to 8.2 CFU/g depending on the strain, and since the lower detection limit in this experiment was 2 log CFU/g, this corresponds to the maximum

reduction that could be determined. This level of reduction could be achieved at 500 MPa for a process temperature of 40 °C and 5 °C, and at 600 MPa for a process temperature of 25 °C. The inactivation by HP of spoilage organisms in different meat products was also studied by other authors. Garriga et al. (2002) reported that *Lb. sakei* CTC746 and *Lc. carnosum* CTC747 decreased by more than 6 log units at 400 MPa (17 °C, 10 min) in a meat model system, which was made of cooked ham blended with distilled water (1:3). In later work from the same research group, complete inactivation (in this case > 4.6 log units) of the same bacteria in sliced cooked ham was reported upon treatment at 600 MPa at a higher temperature (31 °C, 6 min) (Jofré et al., 2009). Thus, our current findings together with this earlier work allow to conclude that treatment at 600 MPa at 5-25 °C should suffice for a 6 log inactivation of these specific spoilage organisms. Treatment at 40 °C was not further considered because most currently available industrial HP machines do not have heating, and because treatment at this temperature would not be desirable in practice because it is too favourable for bacterial growth.

Since the cooked ham model product initially contained approximately 1 log CFU/g of lactic acid bacteria, and each package contained 100 g of product, a 6 log reduction should reduce the occurrence of lactic acid bacteria to one cell per thousand packages on average. The results of the pilot scale experiment show that treatment at 600 MPa (10 °C, 10 min) indeed prevented outgrowth of lactic acid bacteria in all packages during the entire storage period of 84 d at 7 °C (Table 2). However, spoilage was not completely prevented, since several packages showed an increase in aerobic mesophilic count. The identity of the implicated bacteria is not clear, but based on more specific plating approaches, they appear to be different from *Enterobacteriaceae*, sulphite reducing anaerobes, *Brochothrix* spp., and aerobic or anaerobic sporeformers. Further work will be needed to identify the nature of these bacteria.

Interestingly, the addition of either caprylic acid (0.15%) or Purasal® (2.5%) in combination with HP treatment at 600 MPa completely prevented this incidental spoilage and in fact any microbial outgrowth during the entire period of 84 d in our pilot scale experiment. The use of either additive alone, without HP treatment, was largely insufficient, and the results thus clearly illustrate the power of combining HP treatment with (natural) antimicrobials as an intrinsic hurdle. More specifically, using only HP treatment, the first package with a significantly (2 log) increased bacterial count appeared after 40 d. Since three packages were analyzed at every time point in the pilot scale experiment, this means that a total of 18 packages had been analyzed at that stage of storage. Using only caprylic acid or Purasal® (no HP), the first package with a 2 log increase was observed respectively after 33 d and 26 d, corresponding to 15 and 12 observed packages in total. In contrast, no increase in microbial count was observed over the entire 84 d period in any of the 72 packages analyzed that were treated with the combination of HP with either one of the additives. This is quite a remarkable achievement, in particular since the storage temperature was relatively challenging (7 °C). Evidently, further work should be done to optimize the combined treatment, both in terms of type and concentration of natural additives used. We used caprylic acid and Purasal® in our work because these compounds have previously been studied and shown to have some effectiveness as antimicrobials in meat products (Blom et al., 1997, Mbandi, & Shelef, 2001; Mbandi, & Shelef, 2002; Burnett, Chopskie, Podtburg, Gutzmann, Gilbreth, & Bodnaruk, 2007; Marcos et al., 2008).

In spite of some differences in experimental setup and parameters that have been analyzed, comparison of our results with those of pilot scale shelf-life studies in the literature leads to roughly similar conclusions concerning the efficacy of HP treatment without additional natural antimicrobials for extending the shelf-life of cooked ham. Garriga et al. (2004) treated cooked

ham at 600 MPa (6 min, 31 °C) and analyzed its microbiological quality during storage at 4 °C. The total aerobic count was reduced with more than 0.45 log units to < 2 log CFU/g by the process, but reached 5.6 log CFU/g after 90 d of storage, compared to 8.63 log CFU/g in the untreated ham. After pressurization and up to 60 days LAB stayed low during storage at 4 °C. However after 90 days already 5.7 and 8.57 log CFU/g LAB were detected in the HP treated and the untreated ham respectively. Yeasts and *Enterobacteriaceae* remained below detection limit (< 1 log CFU/g) during 120 d after HP treatment. In a different study, using a lower pressure and process temperature (400 MPa, 5-20 min, 7 °C), the shelf-life of cooked ham could only be increased by 35 days at 2 °C (López-Caballero et al., 1999). Pietrzak et al. (2007) confirmed that HP treatment at 600 MPa, (in this case at 20 °C for 10 min) reduced the mesophilic and psychrophilic count of cooked pork ham to a level that was initially undetectable, but that again surpassed the detection level after some weeks of cold storage (10^2-10^3 CFU/g after 8 weeks). The major conclusion from these studies as well as from our own data is that HP treatment at 600 MPa for up to 10 min under refrigerated or ambient temperature conditions can considerably reduce the spoilage microbiota of cooked ham and thus extend its shelf-life, but fails to eliminate a small residual level of bacteria, which can spoil the product if they are able to grow out. Interestingly, sporeforming bacteria have not emerged as spoilage microbiota of HP treated cooked ham in any of these studies including our own, although HP treatment, even at 600 MPa, does not efficiently inactivate spores. This probably indicates that the non-sporeforming survivors are more competitive and suppress outgrowth of the sporeforming survivors.

Besides spoilage organisms, we also studied inactivation of surrogate strains for the pathogens *Escherichia coli* O157:H7 and *Listeria monocytogenes* which are of major concern in cooked meat products. Both organisms displayed a high strain variability in HP inactivation. For *E. coli*

ATCC43888 and its HP resistant derivative *E. coli* LMM1080, depending on the process temperature, a 6 log reduction could be achieved at 400 - 600 MPa, i.e. within the reach of the available industrial HP equipment. The HP resistant strain *E. coli* LMM1010 could also be completely inactivated (≥ 5.5 log reduction) at process temperatures of 25 °C and 40 °C, but was reduced by no more than about 2 log at 5 °C, even at a pressure up to 700 MPa. Since industrial HP treatment of perishable products like cooked ham will normally be performed in refrigerated conditions, strains like LMM1010 that are extremely HP resistant under these conditions are a serious concern. The HP resistance of strain LMM1010 has been evolved by selection in the laboratory, but natural *E. coli* strains approaching this level of HP resistance have been described (Benito, Ventoura, Casadei, Robinson, & Mackey, 1999; Pagán, & Mackey, 2000). A high strain variation in HP resistance of *E. coli* was also observed by other authors (Patterson, Quinn, Simpson, & Gilmour, A., 1995; Alpas, Kalchayanand, Bozoglu, Sikes, Dunne, & Ray B., 1999). However, studies in meat products appear to have been conducted only with relatively HP sensitive strains, reporting reductions of 6 log at 405 MPa (25 °C, 10 min) in pork slurries (Shigehisa, Ohmori, Saito, Taji, & Hayashi, 1991), 4.5 log (complete inactivation) at 400 MPa (17 °C, 10 min) in a meat model system (Garriga et al., 2002), and ≥ 2.5 log (complete inactivation) at 600 MPa (31 °C, 6 min) in cooked ham (Jofré et al., 2009). For the two strains of *L. innocua* used in our work, the difference in HP inactivation was smaller, particularly at 5 and 25 °C, and, importantly, both strains could be reduced by 5 to 6 log at all temperatures at 600 MPa. Other studies with cooked ham reported a 6 log reduction of two *L. monocytogenes* strains after a treatment of 400 MPa (17 °C, 10 min) (Garriga et al., 2002), and a 2.5 log reduction (complete inactivation) of a cocktail of three *L. monocytogenes* strains at 600 MPa (10 °C, 5 min) (Jofré et al., 2009). Marcos et al. (2008) also demonstrated that lactate – diacetate (2.5%)

could be used to suppress outgrowth of *L. monocytogenes* in cooked ham after a relatively mild HP treatment (400 MPa, 17 °C, 10 min).

Finally, we also studied some non-microbiological quality attributes of the HP treated cooked ham. A significant increase of syneresis was observed after HP treatment, which remained stable during cold storage. Enhancement of drip loss was also observed by Pietrzak et al. (2007) in HP treated (600 MPa, 10 min, 20 °C) pork ham, but López-Caballero et al. (1999) found no changes in water binding of ham treated a 400 MPa (5-20 min, 7 °C) during storage at 2 °C. This difference could be due to the lower pressure used in the latter study, or to differences in composition. It is also of importance to mention that the packages with ham slices were stored vertically in our study so that any fluid exudate is collected at the bottom of the package. However, we observed that almost all of the fluid that was lost immediately after HP treatment was reabsorbed after one to two days when the packages were stored in horizontal position. Generally no important colour changes were observed after HP treatment. During storage the cooked ham turned greyish, but there were no significant differences between the untreated and the HP treated ham. This confirms the findings of other authors (Garriga et al., 2004; Pietrzak et al., 2007). The pH-value of both the untreated and the HP treated meat remained relatively stable during storage, in spite of the development of LAB or other spoilage bacteria. A similar observation was done by López-Caballero et al. (1999). Finally, sensory analysis by a trained taste panel did not reveal any differences between the untreated and HP treated samples, confirming earlier findings (Pietrzak et al., 2007). However, the presence of the antimicrobials had a strong negative influence on the flavour and aroma. Further work will be needed to select antimicrobials and optimize their concentration in order to reconcile the required microbial stability and safety with sensorial quality.

In conclusion, we have demonstrated the potential of HP to extend the shelf-life of cooked ham. Treatment at 600 MPa (10 °C, 10 min) delayed microbial spoilage by about 9 weeks without changing sensorial quality attributes of the product. Furthermore, we demonstrated that combining HP treatment with natural antimicrobials is a powerful approach to achieve a further shelf-life extension, provided that sensorially acceptable formulations are developed.

5. Acknowledgments

This work was conducted within the framework of a Flanders' Food project (Brussels, Belgium).

6. Tables

Table 1: Overview of HP studies conducted on cooked ham

Footnote to Table 1

[a] challenge study

[b] naturally occuring microbiota

Table 2: Microbial counts (log CFU/g) of HP treated (600 MPa, 10 °C, 10 min) and untreated cooked ham model products with or without Purasal® (2.5%) or caprylic acid (0.15%) during cold storage (7 °C). Each data point is an average ± standard deviation from three individual packages.

Footnote to Table 2

[a*] = difference between the lowest and the highest count for the three analyzed packages was more than 3 log; [b]ND= not determined.

7. Figures

Figure 1: Survivors (log CFU/g) of *B. thermosphacta* M1 (■), *C. divergens* M6 (■), *Lc. carnosum* M3 (■) and *Lb. sakei* DSM6333 (□) in inoculated cooked ham model product after HP treatment at A) 5, B) 25 and C) 40 °C for 10 minutes. The average for three separate treatments is shown, with standard deviation indicated as error bars.

Figure 2: Survivors (log CFU/g) of *E. coli* O157:H7 ATCC43888 (■), *E. coli* O157:H7 LMM1080 (■), *E. coli* LMM1010 (⊟); *L. innocua* CIP80.12 (■) and *L. innocua* CIP80.11 (□) in inoculated cooked ham model product after HP treatment at A) 5, B) 25 and C) 40 °C for 10 minutes. The average for three separate treatments is shown, with standard deviations indicated as error bars.

Figure 3: Effect of HP on the syneresis of cooked ham model product. 0.1 MPa without additives (■), 0.1 MPa with caprylic acid (■), 0.1 MPa with Purasal® (□), 600 MPa without additives (x), 600 MPa with caprylic acid (Δ) and 600 MPa with Purasal® (o). The results of three measurements are given, with standard deviation indicated as error bars.

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Table 1: Overview of HP studies conducted on cooked ham

Experimental conditions				Output variables			
Pressure	**Antimicrobial**	**Duration of shelf-life study**	**T storage (°C)**	**(surrogate) pathogens**	**Spoilage organisms**	**Others**	**Reference**
200 MPa/5-20 min/7 °C 400 MPa/5-20 min/7 °C		35 d	2 °C		Total viable count[b] Lactic acid bacteria *Brochothrix* spp *Enterobacteriaceae* Baird Parker flora	Water binding pH	López-Caballero et al., 1999
600 MPa/6 min/31 °C		120 d	4 °C		Aerobic total count[b] Psychrotrophs Lactic acid bacteria Yeasts *Enterobacteriaceae*		Garriga et al., 2004
400 MPa/10 min/30°C		126 d	2, 12, 7°C		Lactic acid bacteria[b] Total viable count *Enterobacteriaceae*	Biogenic amines Free amino acids	Ruiz-Capillas, Carballo, & Colmenero, 2007
200 MPa/5-15 min/27 °C 300 MPa/10 min/27 °C 400 MPa/5-15 min/27 °C		85 d	8 °C		Lactic acid bacteria[b]		Slongo, Rosenthal, Camargo, Deliza, Mathias, & de Aragão, 2009
600 MPa/10 min/20 °C		8 we	4-6 °C	*Salmonella*[b] *S. aureus*	Enterococci[b] Mesophilic count Psychrotrophs	Colour Taste Water binding	Pietrzak et al., 2007
600 MPa/ 6 min/31°C		120 d	4°C	*L. monocytogenes*[a] *S. aureus* *Salmonella* *E. coli* *Campylobacter jejuni* *Y. enterocolitica*	*Lb. sakei*[a] *Lc. carnosum* *Debaryomyces hansenii*		Jofré et al., 2009
400 MPa/ 10 min/17°C	Enterocins A,B Sakacin K Pediocin AcH	60 d	4°C	*E. coli*[a] *Salmonella* *L. monocytogenes* *S. carnosus*	*Lb. sakei*[a] *Lc. carnosum*		Garriga et al., 2002

	Nisin			*S. aureus*			
400 MPa/10 min/17°C	Lactate Nisin	84 d	1°C 6°C	*L. monocytogenes*[a] *Salmonella*			Aymerich et al., 2005
600 MPa/5 min/10°C	Lactate Nisin-lactate Nisin	90 d	1°C 6°C	*L. monocytogenes*[a] *S. aureus* *Salmonella*			Jofré et al.., 2008
400 MPa/10 min/17°C	Lactate-diactetate Enterocins	84 d	1°C 6°C	*L. monocytogenes*[a]			Marcos et al., 2008
100-700 MPa/10 min/ 5,25, 40°C (lab-scale) 600 MPa/10 min/10°C (pilot-scale)	Lactate – diacetate Caprylic acid	84 days	7°C	*E. coli* LMM1010[a] *E. coli* LMM1080 *E. coli* ATCC43888 *L. innocua* CIP80.11 *L.innocua* CIP80.12	*B. thermosphacta* M1[a] *L. carnosum* M3[a] *C. divergens* M6[a] aerobic mesophilic count[b] lactic acid bacteria[b] anaerobic mesophilic bacteria[b] *Brochothrix* spp.[b] sulfite reducing clostridia[b] *Enterobacteriaceae*[b]	Colour pH taste water binding	Current study

Table 2: Microbial counts (log CFU/g) of HP treated (600 MPa, 10 °C, 10 min) and untreated cooked ham model products with or without Purasal® (2.5%) or caprylic acid (0.15%) during cold storage (7 °C). Each data point is an average ± standard deviation from three individual packages.

		Time (days)											
		1	**8**	**15**	**26**	**33**	**40**	**45**	**53**	**59**	**68**	**73**	**84**
Aerobic mesophilic count	no additives, 0.1 MPa	1.2±0.2	1.0±0.0	2.0±2.1*[a]	1.0±0.2	2.7±2.0	6.2±1.4	7.7±1.8*	ND[b]	ND	ND	ND	ND
	no additives, 600 MPa	0.7±0.2	<0.5	1.0±0.4	0.6±0.3	<0.5	5.4±0.3	2.5±1.9	2.3±2.9*	5.1±0.8	4.2±3.3	0.7±0.2	<0.5
	caprylic acid, 0,1 MPa	1.0±0.5	0.6±0.3	0.6±0.3	0.9±0.8*	3.0±2.4*	4.3±2.9*	4.5±3.5*	ND	ND	ND	ND	ND
	caprylic acid, 600 MPa	1.0±0.3	1.3±0.3	0.9±0.4	0.7±0.2	0.9±0.4	0.9±0.4	0.8±0.3	0.8±0.6	0.9±0.5	0.9±0.4	0.8±0.5	0.8±0.5
	Purasal®, 0.1 MPa	1.0±0.4	1.4±0.3	1.4±0.9	4.7±0.4	4.1±3.2*	6.6±1.2	ND	ND	ND	ND	ND	ND
	Purasal®, 600 MPa	0.7±0.4	0.8±0.4	0.9±0.4	0.6±0.2	0.7±0.2	0.7±0.2	0.7±0.2	1.1±0.4	0.9±0.5	1.0±0.4	0.6±0.3	<0.5
Lactic acid bacteria	no additives, 0.1 MPa	1.2±0.2	1.9±0.8	2.2±2.9*	2.7±2.0*	1.4±1.7*	3.6±2.9*	7.2±2.1*	ND	ND	ND	ND	ND
	no additives, 600 MPa	1.0±0.2	0.6±0.2	0.6±0.3	0.6±0.2	<0.5	<0.5	0.7±0.4	<0.5	0.6±0.2	<0.5	<0.5	0.7±0.4
	caprylic acid, 0,1 MPa	1.4±0.5	0.6±0.2	0.9±0.4	0.8±0.0	3.1±2.2	0.6±0.3	4.6±3.1	ND	ND	ND	ND	ND
	caprylic acid, 600 MPa	1.1±0.5	0.9±0.5	0.6±0.2	0.6±0.3	<0.5	0.9±0.4	1.1±0.7	0.9±0.5	1.1±0.2	0.9±0.5	0.7±0.4	0.6±0.3
	Purasal®, 0.1 MPa	1.1±0.6	0.8±0.3	2.8±2.4*	4.9±0.3	2.3±1.6	2.3±3.2*	0.7±0.2	ND	ND	ND	ND	ND
	Purasal®, 600 MPa	0.8±0.4	<0.5	0.7±0.4	<0.5	0.7±0.4	<0.5	1.1±0.4	0.8±0.6	<0.5	0.9±0.4	0.6±0.2	1.1±0.0

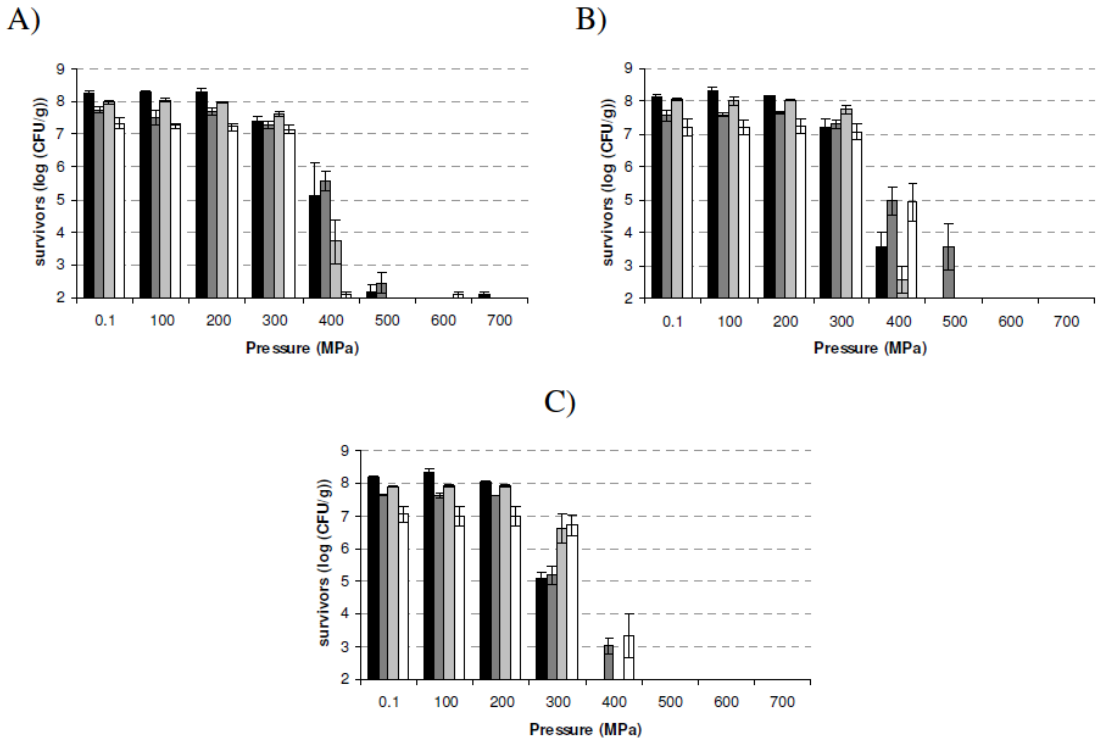

Fig. 1

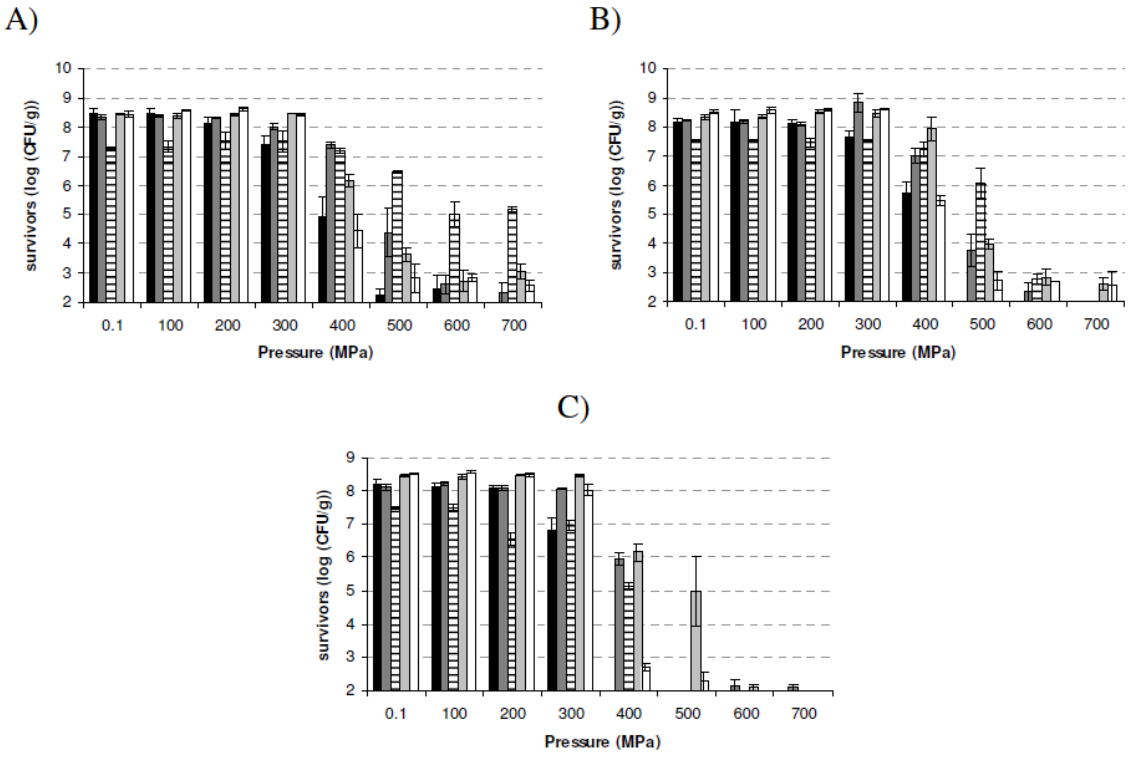

Fig. 2

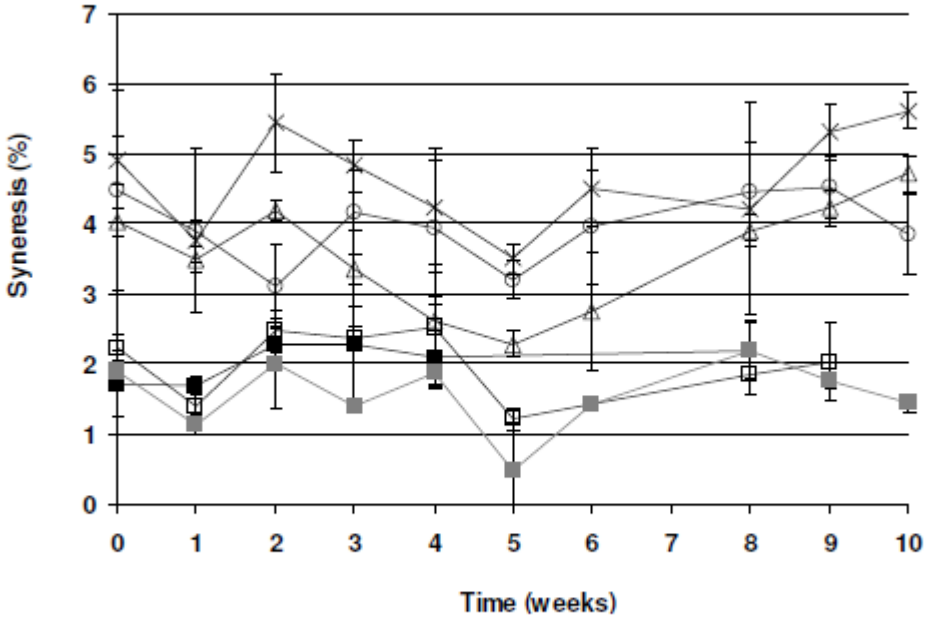

Fig. 3

Highlights

- 5-D reduction of pathogens and spoilage bacteria in cooked ham at ≥ 600 MPa at ≥ 25 °C
- Combined HP treatment and antimicrobials : shelf-life at least 84 days at 7°C
- Increased drip loss after HP treatment, no effect on other quality aspects