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Efficiency of high hydrostatic pressure at 600 MPa against food-borne microorganisms by challenge tests on convenience meat products

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ABSTRACT

The food-borne pathogens *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*, *Yersinia enterocolitica* and *Campylobacter jejuni*, and the spoilage lactic acid bacteria (LAB), *Escherichia coli* and the yeast *Debaryomyces hansenii* were inoculated on slices of cooked ham, dry cured ham and marinated beef loin. During storage at 4 °C, *L. monocytogenes* and LAB increased up to 3.5 log units while the other species, unable to grow under refrigeration, continued at the spiking level. The application of a 600 MPa treatment effectively inactivated most of the microorganisms, the counts of which, except for LAB that increased in cooked ham and in beef loin, progressively decreased or maintained below the detection limit during the whole storage (120 days at 4 °C).

1. Introduction

Technological advances developed by the food industry have enabled the control of microbial hazards and, in some countries, have led to a reduction of food-borne diseases. However, the impact of food-borne pathogens on health is still very important although the nature of the implicated food and food-borne illnesses has changed during the last decade. While *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* have been associated with food-borne illness for decades, consumer demand for freshly prepared foods has been related to the emergence of pathogens such as *Yersinia enterocolitica*, *Campylobacter* spp., *Listeria monocytogenes* and *Escherichia coli* O157 in the last decades (Borch & Arinder, 2002; Tauxe, 2002). In recent years, *Salmonella* and *Campylobacter* have been the most common agents responsible for outbreaks in the EU (53.9% and 6.9% of all outbreaks, respectively). Other important causes of food-borne outbreaks are food-borne viruses (10.2% of all reported outbreaks), bacterial toxins (from *Staphylococcus* spp. (4.1%), *Clostridium* spp. (1.4%) and *Bacillus* spp. (1.3%), pathogenic *E. coli* (0.8%), *Shigella* (0.6%) and *L. monocytogenes* (0.2%) (European Food Safety Authority, 2007)).

High hydrostatic pressure (HHP) is an emerging technology that is receiving a great deal of attention as shown by research and commercialization efforts performed worldwide. Within the meat sector, HHP offers a valuable alternative to the thermal pasteurization to be applied after product manufacture, especially for convenience products (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). Nowadays, available industrial HHP equipment can reach pressures of 600 MPa. At this pressure level, inactivation of bacteria such as *Salmonella* and *L. monocytogenes* has been shown to be highly effective. However, only limited information is available for other microorganisms and/or food products (Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004; Jofré, Aymerich, Monfort, & Garriga, 2008; Jofré, Garriga, & Aymerich, 2008). In addition, the antimicrobial effect of HHP can be affected by the food composition, including the pH, water activity (a_w) and redox potential (Gao, Ju, & Wu, 2007). In cooked and dry cured ham, it has been shown that when HHP at 600 MPa is combined with bacteriocins, different efficiencies are obtained depending on the meat product (Jofré et al., 2008).

The aim of this study was to evaluate the effect of the application of an HHP treatment of 600 MPa to three convenience food products (i.e. sliced cooked ham, sliced dry cured ham and marinated beef loin) independently challenged with food-borne pathogens (*L. monocytogenes*, *Salmonella enterica*, *S. aureus*, *Y. enterocolitica* and *Campylobacter jejuni*), spoilage microorganisms (slime producing LAB or the yeast *Debaryomyces hansenii*) and the hygiene indicator *E. coli*. The effect of pressure after the treatment and during storage for 120 days at 4 °C was assessed.

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2. Materials and methods

2.1. Manufacture of meat products

Cooked ham ingredients were (in g/kg): pork ham 884, water 95, sodium chloride 17.8, carrageenan 1.0, sodium citrate 1.5, sodium ascorbate 0.5, sodium nitrite 0.12 and spices 0.12. Meat was tenderised and injected with brine containing the ingredients. Cooking was performed in an oven at 67 °C until core temperature reached 65 °C. Before slicing ham was stored under refrigeration (3 °C) for 48 h. a_w of final product = 0.982, pH = 6.11.

Dry cured ham ingredients were (in g/kg): pork ham 950, sodium chloride 46, dextrose 4 and potassium nitrate 0.2. Meat was dry salted with the mixture of ingredients and left to rest in horizontal layers for salt diffusion in a cold room for 14 days at 0–4 °C and 85–95% of relative humidity (RH). Afterwards, a 40 day post-salting period was performed at 2–6 °C and 70–95% RH and a 40 day drying period was carried out at increasing temperatures from 6 °C to 14 °C and at 70–95% RH. Ageing–maturation (7 months) was performed at increasing temperatures from 14 °C to 34 °C and at 60–80% RH. a_w of final product = 0.918, pH = 5.88.

Marinated beef loin ingredients were (in g/kg): beef loin 943, water 44, sodium chloride 10, sodium tri-polyphosphate 2, sodium ascorbate 0.6 and sodium nitrite 0.2. Meat was marinated with brine containing all the ingredients. After vacuum-packing, samples were left to rest in a cold room for 48–72 h. a_w of final product = 0.987, pH = 6.26.

2.2. Slicing, inoculation and packaging

The products were sliced (1.5 mm) and the following species (a mixture of strains) were independently spiked between 2 slices of product at a level of *ca.* 3.5 log CFU/g: *S. enterica* subsp. *enterica* (*S.* London CTC1003, *S.* Schwarzengrund CTC1015 and *S.* Derby CTC1022); *L. monocytogenes* (CTC1010, CTC1011 and CTC1034); *S. aureus* (CTC1008, CTC1019 and CTC1021); *E. coli* (CTC1007, CTC1018 and CTC1023); *C. jejuni* (CTC1032); *Y. enterocolitica* (CTC1061, CTC1062 and CTC1064); slime producing LAB (*Lactobacillus sakei* CTC746 and *Leuconostoc carnosum* CTC747) and *D. hansenii* CTC5001. All strains belong to our own collection and were from animal or food origin.

Slices of approximately 40 g were vacuum-packed in pairs into 137 × 250 mm packs using Multivac MP Darfresh equipment (Germany). Bottom film was polystyrene–EVOH based (oxygen permeability 2 cm^3/m^2, 24 h, 1 bar) and upper film was polyethylene–EVOH based (oxygen permeability 2 cm^3/m^2, 24 h, 1 bar; water vapour permeability <7 g/24 h, m^2) (Darfresh system from Cryovac; Grace S.A., Sant Boi de Llobregat, Spain). The packaged sliced products (cooked ham, dry cured ham and marinated beef loin) were stored for 24 h at 4 °C before the HHP treatment.

2.3. High-pressure treatment and storage

Half of the samples of each type of product were treated at 600 MPa for 6 min at 31 °C in an industrial hydrostatic pressurization unit QFP 35L-600-1 (Flow Pressure Systems Vasteras AB, Sweden). Come up time was 125 s and pressure release was immediate. Pressurized and non-pressurized samples were stored at 4 °C for 120 days.

2.4. Microbiological analysis

Three different packages were analyzed for each product on day 0 (before HHP treatment), 2, 30, 60 and 120 of storage. For plate counting, slices were minced and 25 g of sample were diluted 1/10 in 0.01 g/l Bacto Peptone (Difco Laboratories, Detroit, MI, USA) with 0.085 g/l NaCl (Merck, Darmstadt, Germany) and homogenized in a Stomacher Labblender (model 400, Cooke Laboratory Products, Alexandria, VA, USA). The homogenate was serially diluted and plated onto appropriate culture media. LAB in de Man, Rogosa & Sharpe agar (MRS, Difco Laboratories) double-layered plates incubated at 30 °C for 3 days in anaerobiosis. Yeasts in Sabouraud Dextrose 0.2 g/l agar (SDA, Merck) poured plates at 25 °C for 5 days. *E. coli* in Coli ID (bioMérieux, Marcy l'Etoile, France) double-layered plates at 37 °C for 48 h; typical colonies were confirmed by API 20E (bioMérieux). *S. aureus* on Baird–Parker (Difco Laboratories) spread plates at 37 °C for 48 h, and typical colonies were confirmed by latex agglutination test (Slidex Staph Plus, bioMérieux). *L. monocytogenes* and *Salmonella* spp. were counted on Palcam agar (Merck) spread plates at 30 °C for 48 h and Brilliant Green Agar (BGA, Difco Laboratories) spread plates at 37 °C for 24 h, respectively.

Additionally, in samples with pathogen counts below the quantification limit, the presence/absence of *L. monocytogenes* was investigated by preenrichment of 10 g of sample in Listeria enrichment broth (UVMI, Oxoid, Basingstoke, Hampshire, England) at 30 °C for 24 h followed by enrichment in UVMII at 30 °C for 24 h and selective isolation in Palcam agar. Suspected colonies were confirmed by PCR (Klein & Juneja, 1997). The presence/absence of *Salmonella* spp. (in 25 g), *Campylobacter* spp. (in 10 g) and *Y. enterocolitica* (in 10 g) was investigated according to ISO 6579, ISO 10272 and ISO 10273, respectively. Typical colonies were confirmed by API 20E, for *Salmonella* spp. and *Y. enterocolitica*, and API Campy, for *Campylobacter* spp. (bioMérieux).

2.5. Physico-chemical analysis

The pH was measured directly in the samples using a Crison penetration 52–32 electrode connected to a Crison Basic 20 pH-meter (Crison Instruments S.A., Alella, Spain). Water activity (a_w) measurement was carried out using a Novasina Thermoconstanter TH-500 (Novasina, Switzerland) at 25 °C.

2.6. Statistical analysis

Data were analyzed by analysis of the variance (ANOVA), followed by Tukey's test at the 0.05 level of probability using the Statistica 7.0 software (Statsoft, Tulsa, UK).

3. Results

3.1. Evolution of microorganisms in non-pressurized meat products under refrigeration

The behaviour of the challenged microorganisms during the storage of meat products is shown in Fig. 1. Among pathogens, only *L. monocytogenes* could grow in cooked ham but not in the other products, in which the pathogen remained at the initial spiked level throughout the 120 days of storage at 4 °C. *Salmonella* and *S. aureus* continued at the initial level during the whole storage of all the meat products. In dry cured ham and beef loin the initial spiked level of *Y. enterocolitica* decreased below the detection limit (presence in 10 g) during the first 30 days and continued at this level during the whole storage. In cooked ham, absence of *Y. enterocolitica* was only recorded after 120 days of storage. *C. jejuni*, in contrast, recorded presence in 10 g during the entire storage time (not shown).

Regarding the spoilage/hygiene related microorganisms, *E. coli* continued at the spiking level for 120 days in all meat products. In contrast, total LAB (spiked and endogenous) showed a 2.9 and