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NaCl-free processing, acidification, smoking and high pressure: Effects on growth of *Listeria monocytogenes* and *Salmonella enterica* in QDS processed® dry-cured ham

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ABSTRACT

To evaluate the food safety effect of NaCl-free processing, acidification, smoking and high pressure in QDS processed® dry-cured ham, 3 ham types (non-acidified smoked, acidified, and acidified smoked) were produced according to a standard (-S) and a new NaCl-free (-F) process. Slices were spiked with *Listeria monocytogenes* and *Salmonella enterica* (three strains each, <2 log CFU/g), dried by the QDS process®, vacuum packed, high pressure treated at 600 MPa and stored under refrigeration for 112 days. Results of the challenge test showed that *L. monocytogenes* could only be eliminated from acidified smoked (AS) -S and -F processed ham slices at the end of storage, while *S. enterica* was present in all non-pressurized slices. The safest hams were those pressurized, especially AS-S hams, where *L. monocytogenes* was eliminated from 25 g of product immediately after HP treatment and *S. enterica* after 14 days. Compared with standard processing, NaCl-free processing showed lower levels of pathogens in non-pressurized slices but their elimination was delayed in pressurized ham slices.

1. Introduction

Food safety and stability of traditional dry-cured meat products are based on a number of hurdles (pH, water activity (a_w), nitrite), which assure a long shelf-life through their combined effect (Leistner, 2000; Reynolds, Harrison, Rose-Morrow & Lyon, 2001). They also prevent the growth of pathogens such as *Listeria monocytogenes* and *Salmonella* spp., which are of concern in ready-to-eat (RTE) products (European Food Safety Authority, 2013). In dry-cured meat products the most important hurdle is the low a_w, achieved through the addition of salts and long ripening times. However, nowadays a_w values found in sliced and vacuum packed dry-cured meat products are often higher than 0.92 (Hereu, 2009). Further, the fact that slicing represents a possible cross-contamination source for meat products (Talon et al., 2007) and the tendency to reduce the NaCl content (WHO, 2007) require a redesigning in dry-cured ham manufacturing and proper food safety investigation.

From an investment point of view, ripening is the most time and energy consuming step, which can last from a few weeks up to years. To accelerate the process various strategies have been described (Arnau, Serra, Comaposada, Gou & Garriga, 2007), among them the Quick Dry Slice process® (QDS process®) based on the patented technology from Comaposada, Arnau, Gou & Monfort (2004). This innovative process facilitates the reduction of the drying period of sliced products by direct drying of slices in a continuous system. Additionally, better control of processing and product quality provides a great flexibility in production planning, an aspect which is of great importance for product development.

From a food safety point of view, the inclusion of antimicrobial ingredients (e.g. lactate) and/or additional (fermentation/acidification and smoking) or alternative techniques such as high pressure (HP) processing may also be of great interest in the development of safe new products. The antimicrobial effect of lactate has been demonstrated in different meat products against *Salmonella* spp. and especially *L. monocytogenes* (Jofré, Garriga & Aymerich, 2008; Mbandi & Shelef, 2002; Miller & Acuff, 1994; Stekelenburg, 2003). Fermentation has been described to improve product stability, flavour and texture in dry-cured meat products (e.g. fermented sausages) and smoking has antibacterial and

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fungicide properties (Girard, 1988; Toth & Potthast, 1984). HP processing, a non-thermal food preservation technology, can be used for microbiological safety improvement and shelf life extension of RTE foods. For dry-cured ham, a recently published model for HP inactivation showed that pressurization at 613 MPa for 5 min was sufficient to achieve the *L. monocytogenes* US "zero tolerance" policy (Bover-Cid, Belletti, Garriga & Aymerich, 2011) considering the low contamination levels and the inability of *L. monocytogenes* to grow in this product. For *Salmonella enterica*, a significantly reducing effect after pressurization at 600 MPa has already been described (Bover-Cid, Belletti, Garriga & Aymerich, 2012; Jofré, Aymerich, Grèbol & Garriga, 2009; Stollewerk, Jofré, Comaposada, Arnau & Garriga, 2012a).

As postulated by the hurdle technology, the combination of different preservative factors is more efficient for controlling microorganisms in food than using individual hurdles (Leistner, 2007). Based on this technology, the aim of the present study was to evaluate through a challenge test the fate of *L. monocytogenes* and *S. enterica* spiked on QDS process® dried slices of dry-cured ham, manufactured with and without NaCl, acidification, smoking and pressurization.

2. Materials and methods

2.1. Manufacture of dry-cured hams and partial drying

Three ham types (non-acidified smoked (NS), acidified (A) and acidified smoked (AS)) were manufactured following different salting processes (dry salting or brine injection). Furthermore, composition and manufacture of hams was adapted to the production process (standard (-S) and NaCl-free (-F)), based on previous sensorial results (Arnau, Comaposada, Serra, Bernardo & Lagares, 2011). The main differences between -S and -F processed hams included the substitution of NaCl by KCl and potassium lactate and the addition of more sugars to compensate the bitter taste of KCl and potassium lactate (Gou, Guerrero, Gelabert, & Arnau, 1996). Acidification to a pH of approximately 5.2 in A-S and AS-S hams was achieved by lactic acid bacteria (LAB) fermentation, while calculated amounts of gluconodeltalactone (GDL) were applied to A-F and AS-F hams to produce acidification because the addition of lactate can affect the growth of LAB.

Fig. 1 shows a schematic representation of the manufacturing process. All types of ham were elaborated from commercial raw boned hams trimmed of skin and subcutaneous fat with a $pH_{24} < 6.0$ in *semimembranosus* muscle. Non-acidified smoked hams (NS-S and NS-F) were salted directly in the massaging unit with the addition of the ingredients (Table 1). The curing period was 48 h for NS-S and 72 h for NS-F hams to assure ingredient absorption. In the second massage, to help binding, 3 g/kg of transglutaminase (Denatex 100pur, Activa WM, Ajinomoto®, Impex Química, SA, Barcelona, Spain) and 3.5 g/kg of sodium lactate, were added, which were substituted by equal molar concentrations of potassium lactate (4 g/kg) in NS-F hams. Non-acidified smoked hams (NS-S and NS-F) were covered with a collagen film and then packed (Fig. 1). Acidified hams with (A-S) and without (A-F) NaCl and acidified smoked hams with (AS-S) and without (AS-F) NaCl were salted by injecting 15 g of brine in 100 g of meat (Table 1) and continuous tumbling (for 25 min at 20 mbar, 4 °C and 4 rpm). This type of salting allowed the addition of starter cultures (Table 1). Subsequently hams were wrapped in an elastic mesh (Euronet®-FRA®: Rete Spira AS 30 A 19) and vacuum packed (Cryovac bag CN330, 60 micron, 300 × 600 mm). After pressing, all -F hams were repacked in drying bags (Tublin®, TUB-EX ApS, Taars, Denmark; vapour transmission rate 5000 g/50μ/m^2/24 h (38 °C/50% RH) according to the standard ASTM E 96 BW). The bag was used to avoid brine drip in the first stages of the drying process and to allow additive penetration and liquid evaporation up to a weight loss of 8%. Following, NS and AS hams were smoked for 3 h at 25–30 °C by combustion of beech flakes using an oven (Doleschal, Steyr, Austria) connected to a smoker.

Non-acidified smoked hams (NS-S and NS-F) were subjected to a partial drying process, for 25 days at 5 °C and for 8 days at 12 °C until a final weight loss of 24% was achieved. Continuous ventilation was applied for maintaining relative humidity (RH) at 65%. After manufacturing all the hams were frozen at −20 °C.

For each of the 6 different hams (NS-S, A-S, AS-S, NS-F, A-F, AS-F) a total of eight hams were produced in two independent batches (4 hams per production).

2.2. Slicing, inoculation and QDS drying

Two challenge tests were performed on different days using *Salmonella* spp. and *L. monocytogenes*-free hams. For each challenge test and type of product two hams from two independent batches were sliced. Ham slices (2 mm thick, approximately 35 g/slice) were spiked with a mixture of *L. monocytogenes* (strains CTC1011, CTC1034 and CECT4031) and *S. enterica* (strains Typhimurium GN6, London CTC1003 and Derby CTC1022) at low inoculum levels of 50 CFU/g and 40 CFU/g respectively to simulate a recontamination during slicing (Betts, 2010; CRL/AFSSA, 2008; Hoz, Cambero, Cabeza, Herrero & Ordóñez, 2008; NACMCF, 2010). The mixture was prepared by diluting −80 °C frozen cultures (previously grown overnight in BHI) of each strain in distilled water. The inoculation cocktail (0.2 ml) was spread on the surface of the slices with a Drigalsky spreader until it was completely absorbed.

Drying of ham slices was finished by applying the QDS drying, which was performed by convection of air at 30 °C during approximately 50 min at a RH of 40% until a product water content of 54% was reached, calculated on basis of the water content measured before QDS and the drying weight loss. The maximum temperature of slices during the drying process was 20 °C. Subsequently pairs of slices were vacuum packed in plastic bags of PA/PE (oxygen permeability of 50 cc/m^2 (24 h, 23 °C) and water vapour permeability of 2.6 g/m^2 (24 h, 23 °C, 85% RH), Sacoliva S.L., Castellar de Vallès, Spain) and stored for 12 h at 4 °C until HP was applied.

2.3. High pressure treatment and storage

Half of the samples of each ham type were submitted to a HP treatment of 600 MPa for 5 min at an initial temperature of 13 °C in an industrial hydrostatic pressurization unit (Wave 6000 from NCHiperbaric, Burgos, Spain). The chamber volume was 120 l, the come up time was 3.8 min and the pressure release was almost immediate. Subsequently, treated and non-treated samples were stored under refrigeration at 4 °C for 38 days and afterwards at 8 °C for 74 days, following the temperature profile recommended by guidance documents (AFNOR, 2004; CRL/AFSSA, 2008).

2.4. Microbiological analysis

Sampling was performed after inoculation and periodically (1, 14, 28, 56 and 112 day(s) after drying) during storage under refrigeration. For plate counting, 25 g of the product were diluted 1/10 in BHI broth (Brain heart infusion, DB, NJ, USA) and homogenized in a Masticator Classic (IUL S.A., Barcelona, Spain). Appropriate dilutions of the homogenate were plated onto the following media: Chromogenic Listeria agar (Oxoid Ltd., Basingstoke, England) incubated for 48 h at 37 °C for *L. monocytogenes*;