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.

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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 enterica* 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 & Aymeric, 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...
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 inactivity 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 pH24 < 6.0 in semimembranosus muscle. Non-acidified smoked hams (NS-S and NS-F) were salted directly in the massaging unit (Fig. 1) and continuously 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; vacuum transmission rate 5000 g/50µ/m2/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 flake 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 G6, 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 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 Draisgyl 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/m2/day, 24 h, 23 °C) and water vapour permeability of 2.6 g/m2/day (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;
CHROMagar® *Salmonella* Plus (Scharlab, Barcelona, Spain) incubated for 48 h at 37 °C for *S. enterica*; MRS agar (Merck KGaA, Darmstadt, Germany) incubated for 48–72 h at 30 °C in anaerobiosis for LAB and MSA agar (Mannitol salt phenol-red agar, Merck KGaA) incubated 48–72 h at 30 °C for Gram positive catalase positive cocci (GCC+). When counts of *L. monocytogenes* and *S. enterica* were under 135 mm ∅ plate detection limit (10 CFU/g), presence or absence of viable cells in the enriched homogenates (48 h at 37 °C) was investigated as previously described by Stollewerk et al. (2012a).

![Fig. 1. Schematic representation of manufacturing processes of different ham types.](image)

**Table 1**

Composition of the salting mixture applied to standard (-S) and NaCl-free processed (-F) hams (in g per kg of raw meat).

<table>
<thead>
<tr>
<th>Ingredients (g/kg of meat)</th>
<th>Standard (-S)</th>
<th>NaCl-free (-F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS A and AS</td>
<td>NS A and AS</td>
</tr>
<tr>
<td>NaCl&lt;sup&gt;7&lt;/sup&gt;</td>
<td>28</td>
<td>–</td>
</tr>
<tr>
<td>KCl&lt;sup&gt;7&lt;/sup&gt;</td>
<td>–</td>
<td>15.31</td>
</tr>
<tr>
<td>Potassium lactate (77.8% v/v)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>33.83</td>
</tr>
<tr>
<td>Potassium lactate (60% v/v)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sodium lactate (60% v/v)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Water</td>
<td>–</td>
<td>24.57</td>
</tr>
<tr>
<td>GDL</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Dextrose</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sodium ascorbate</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Potassium nitrite&lt;sup&gt;c&lt;/sup&gt;</td>
<td>–</td>
<td>0.15</td>
</tr>
<tr>
<td>Sodium nitrite&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>0.005</td>
<td>–</td>
</tr>
<tr>
<td>Manganese sulfate</td>
<td>0.009</td>
<td>–</td>
</tr>
<tr>
<td>Starter culture (Lyocarni SXH-38 Sacco (Activa) <em>S. xylosus</em></td>
<td>–</td>
<td>0.15</td>
</tr>
<tr>
<td>Starter culture (Grama SE301: <em>L. sakei</em>, <em>S. xylosus</em>, <em>S. carnosus</em>)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Transglutaminase</td>
<td>3&lt;sup&gt;1&lt;/sup&gt;</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>1</sup>Purasan® Hi Pure P Plus, <sup>2</sup>Purasan® Hi Pure P, <sup>3</sup>Purasan S, all from Purac bioquímica, S.A. Montmeló, Spain. Equal molar concentrations of Na<sup>+</sup> and K<sup>+</sup> (superscript 1), potassium lactate and sodium lactate (superscript 2) and nitrite (superscript 3) were used. <sup>4</sup>Ingredients added in the 2nd massage.
2.5. Physico-chemical analysis

pH and aw were determined before drying (day 0), at the beginning (day 1) and end of storage (day 112) as described in Stollewerk et al. (2012a).

2.6. Statistical analysis

Absence of the pathogens in 25 g of product was considered “N = 0” and presence (counts below the plate detection limit (10 CFU/g) but presence in the enriched homogenate) “N = 1”. To allow logarithmic transformation of zero values, log (N + 1) was used. Data was analyzed by analysis of variance (ANOVA), followed by Tukey’s test at the 0.05 level of significance using the Statistica 7.0 software (Statsoft, Tulsa, UK).

3. Results

3.1. Physicochemical parameters

Differences in composition and processing had an impact on the physicochemical properties of the hams. pH values of non-acidified standard hams recorded before QDS drying were 5.45 ± 0.21 in NS-S and 5.75 ± 0.05 in NS-F, however, after drying values increased (Table 2) and were comparable to those found in commercially processed hams during storage. In standard hams, acidification due to fermentation led to significantly lower pH levels when compared to non-acidified (NS) hams, during the whole study. In all NaCl-free processed hams, higher pH levels (ca. 0.3 units, p < 0.05) were observed before drying (Table 2). The QDS-drying process produced a pH increase in all non-pressurized samples, which was significant in NS-S (0.37 units), A-S (0.37 units) and AS-F (0.25 units). During storage, pH values followed the same trend in -S and -F processed NS (decreasing) and A (increasing) hams, whereas a different tendency was observed in AS-S (increasing) and -F (decreasing) processed samples. Slightly lower aw levels were recorded in NS than in acidified hams (0.014 units in -S and 0.006 units in -F hams, Table 3). Regarding manufacture and due to differences in composition of the brine and length of the curing period (1 day shorter in -S hams), initial aw values (before QDS) of -S processed hams were higher than those of -F processed hams (p < 0.05). However, after drying no important differences were found among them and aw values after 112 days of storage did not differ (p > 0.05). The application of a HP treatment did not affect or produced small changes in pH (an increase of 0.14 in NS-F and 0.21 in A-F hams) and aw (changes <0.005 aw units) of dry-cured ham slices (Tables 2 and 3).

3.2. Technological microbiota

At the time of slicing and spiking with pathogens, similar LAB counts were recorded in non-acidified smoked (NS) and -F hams (ca. 10² CFU/g), while in acidified (A) and in acidified smoked (AS) hams, counts were ca. 2.5 log higher in standard (-S) than in NaCl-free (-F) hams (Fig. 2). During the following storage period of 112 days, LAB levels developed similarly in NS hams (Fig. 2). During the following storage period of 112 days, LAB levels developed similarly in NS hams (Fig. 2). During the following storage period of 112 days, LAB levels developed similarly in NS hams (Fig. 2). During the following storage period of 112 days, LAB levels developed similarly in NS hams (Fig. 2). During the following storage period of 112 days, LAB levels developed similarly in NS hams (Fig. 2). During the following storage period of 112 days, LAB levels developed similarly in NS hams (Fig. 2).
Table 3

<table>
<thead>
<tr>
<th>Manufacturing Standard (-S)</th>
<th>NaCl-free processed (-F)</th>
<th>Ham type</th>
<th>NS</th>
<th>A</th>
<th>AS</th>
<th>NS</th>
<th>A</th>
<th>AS</th>
</tr>
</thead>
</table>
| Before QDS process          | HP-                      | A         | 0.985 ± 0.007<sup>bc</sup> | 0.941 ± 0.014<sup>bc</sup> | 0.949 ± 0.002<sup>bc</sup> | 0.952 ± 0.005<sup>bc</sup> | 0.926 ± 0.007<sup>bc</sup> | 0.926 ± 0.007<sup>bc</sup>
| After QDS drying            | HP+                      | A         | 0.995 ± 0.004<sup>bc</sup> | 0.916 ± 0.004<sup>bc</sup> | 0.913 ± 0.004<sup>bc</sup> | 0.915 ± 0.008<sup>bc</sup> | 0.933 ± 0.003<sup>bc</sup> | 0.933 ± 0.003<sup>bc</sup>
| Days of storage             |                          | A         | 1.12 ± 0.01<sup>bc</sup> | 1.35 ± 0.02<sup>bc</sup> | 1.49 ± 0.02<sup>bc</sup> | 1.54 ± 0.02<sup>bc</sup> | 1.79 ± 0.02<sup>bc</sup> | 1.79 ± 0.02<sup>bc</sup>
|                          |                          | A         | 0.926 ± 0.011<sup>bc</sup> | 0.926 ± 0.011<sup>bc</sup> | 0.926 ± 0.011<sup>bc</sup> | 0.926 ± 0.011<sup>bc</sup> | 0.926 ± 0.011<sup>bc</sup> | 0.926 ± 0.011<sup>bc</sup>

Values are means ± SD (n = 4). For S and F processed hams, significant differences in rows are indicated by different capital letters and significant differences in columns are indicated by different small letters (<p>0.05).

Initial GCC+ counts were 3.2 log lower (<p>0.05) in NS-S than in the other hams (6–6.5 log CFU/g). During storage, differences in behaviour were only observed between GCC+ of NS-S and NS-F hams (Fig. 2), which led to a 1.6 log higher final GCC+ level of NS-S ham (<p>0.05). HP did not affect GCC+ counts in A-S, AS-S and all -F ham slices and counts remained at the initial level or slightly decreased (<p>0.05) during storage. In contrast, an increase of 3.5 log (<p>0.05) to initial levels was observed in HP treated NS-S ham after 112 days.

3.3. Pathogenic microbiota

Dry-cured ham slices were spiked with L. monocytogenes and S. enterica at a level of <2 log CFU/g. Subsequent QDS-drying did not significantly affect the levels of pathogens in any of the -S and -F hams (<p>0.05, Fig. 3, day 1). During refrigerated storage of vacuum packed ham slices L. monocytogenes counts decreased similarly (1.3–1.5 log) in all types of ham. At the end of storage, however, absence in 25 g of product was only recorded in AS-S and AS-F samples. Comparing non-pressurized -S and -F samples, equal or lower counts were recorded in -F hams during the whole experiment. A HP treatment of 600 MPa had an immediate bactericidal effect of 1.6 log reduction (<p>0.05) in NS-S and A-S and eliminated the pathogen from all AS-S samples, while it took 112 days to achieve the same result in NS-S. Compared to -S ham samples, immediate reductions caused by HP were 0.22, 0.92 and 0.67 log lower in NS-F, A-F and AS-F, respectively. During storage, similarly, L. monocytogenes decreased slower in -F hams and pathogen absence was recorded in A-F and AS-F hams after 56 days. Regarding S. enterica, a similar decrease during storage was observed in all hams (Fig. 3). However, a HP treatment of 600 MPa was necessary to achieve absence in all -S and -F hams during storage. Taken together, S. enterica elimination from pressurized samples (25 g) was achieved faster in acidified and -S hams, for example, after 14 days the pathogen was absent from AS-S slices but its elimination from NS-F required 112 days.

4. Discussion

The decrease of pH and <i>a</i><sub>w</sub> during the ripening phase of dry-cured meat products due to fermentation and drying, respectively, are among the most important factors to assure food safety and stability (Leistner, 2000). New formulations or manufacturing procedures, which imply possible modifications of these factors, must therefore be properly evaluated. In the present study, differences in <i>a</i><sub>w</sub> observed before QDS drying could be attributed to variations in manufacturing processes (type of salting, curing period, ingredient composition, etc.). However, after QDS drying, <i>a</i><sub>w</sub> values of different ham types were equalized to 0.93–0.94 and maintained at similar levels during storage. Hence, regarding described growth limits of L. monocytogenes and Salmonella spp. (0.92 and 0.94, respectively, ICMSF, 1996), theoretically <i>a</i><sub>w</sub> would not prevent their multiplication during storage. It must also be taken into account that similar <i>a</i><sub>w</sub> levels from hams with varying composition have been achieved by different solutes (NaCl vs. KCl – potassium lactate – sugars) and the type of solute has been shown to have an effect on microbial behaviour (Beuchat, 1974; Strong, Foster & Duncan, 1970). Evaluations of food safety as a consequence of the replacement of NaCl by KCl in broth demonstrated that KCl is a direct 1:1 M replacer for the antimicrobial effect of common salt against Aeromonas hydrophila, Enterobacter sakazakii, Shigella flexneri, Yersinia enterocolitica and Staphylococcus aureus (Bidlas & Lambert, 2008). Regarding L. monocytogenes, Bozarias, Skandamis, Anastasiadi & Nychas (2007) demonstrated that NaCl could be replaced by equal-molar concentrations of KCl.
without risking microbiological safety in culture media. van Burik & de Koos (1990) showed that sodium lactate provided better growth inhibition on *Salmonella typhimurium* and *S. aureus* at equal $a_w$ values in broth. Bacterial behaviour in foodstuff, however, cannot be directly extrapolated from studies in broth due to the significant effect of the food matrix composition (Brocklehurst, 2004). The increase of pH during QDS drying was also observed in previous studies on QDS-dried fermented sausages (Comaposada et al., 2010; Stollewerk, Jofré, Comaposada, Arnau & Garriga, 2011; Stollewerk, Jofré, Comaposada, Arnau & Garriga, 2012b) and could be related with the concentrating effect of the drying process.

From a technological point of view, acidification of dry-cured meat products increases hardness when the pH decreases below the isoelectric point of myosin (pI 5.4) (Hamm, 1986). From a food safety point of view, a low pH represents an additional hurdle to the growth of pathogenic and spoilage bacteria and prolongs shelf life (Barbut, 2005). The stabilizing effect of a low pH in meat products is well known and reported from dry fermented sausages (Leistner, 1995). The Mediterranean dry-cured ham production procedure, however, does not include a fermentation step and the naturally occurring pH change is described to be unlikely a major factor in the microbial stability (Reynolds et al., 2001). Nevertheless, the use of starter cultures in hams salted with brine injection has been proposed to accelerate the production process (Jessen, 1995). In the present study, starter cultures were added to A-S and AS-S hams, while in A-F and AS-F hams, due to the possible growth delaying effect of lactate on LAB (Shelef, 1994), GDL together with GCC$^+$ starter were applied to improve flavour and colour. In this context, it has been observed that GDL enhanced the bactericidal effect of lactate on LAB on vacuum packed beef (García Zepeda et al., 1994). In non-acidiﬁed hams (NS hams), where no LAB starter or GDL was applied, endogenous LAB grew during...
manufacturing. Thus, differences in composition caused different levels of LAB between -S and -F acidified hams at the moment of slicing and during subsequent storage. In contrast to the lower LAB levels in -F hams, GCC\textsuperscript{+} starter behaved similar in -S and -F acidified (A and AS) hams and was not affected by the presence of lactate. The application of a starter was necessary to achieve high levels of GCC\textsuperscript{+} in NS-F, while in NS-S the same or higher levels were reached by endogenous GCC\textsuperscript{+} growth during storage.

Acidi\textsuperscript{cation} was among the most important factors affecting the levels of pathogenic bacteria. In general, acidified hams achieved higher proportion of samples with absence of \textit{L. monocytogenes} and \textit{S. enterica}, which indicated that in dry-cured

**Fig. 3.** Behaviour of \textit{L. monocytogenes} and \textit{S. enterica} in non-pressurized (HP\textsuperscript{-}) and high pressure treated (HP\textsuperscript{+}; 600 MPa/5 min/13°C) standard (-S) and NaCl-free (-F) processed dry-cured ham slices and proportions of presence (P)/absence (A) in 25 g of sample (table format) during the 112 days of refrigerated storage. Data (mean and standard deviation) comes from 2 independent experiments performed in duplicate.
ham, pH reduction could provide additional food safety. The anti-
microbial activity of lactate against *L. monocytogenes* has been
demonstrated in various meat products, especially those cooked,
such as frankfurter sausage (Stekelenburg, 2003), beef bologna
(Mbandi & Shelef, 2002), comminuted cooked beef, cooked chicken
roll and pork liver sausage (Shelef, 1994). An enhanced effect of
lactate in combination with GDL was also observed on *L.
monocytogenes* in a cooked cured emulsion type product, when
0.25% GDL +2% lactate was used instead of lactate alone (Juncher
et al., 2000). In contrast, studies on the inhibiting effect of lactate
against *Salmonella* spp. performed on chicken dry fermented sau-
sages, cooked ham and beef bologna only observed poor or no
pathogen inhibition (Deumier & Collignon, 2003; Jofré et al., 2008;
Mbandi & Shelef, 2002). Although in none of the dry-cured hams
*L. monocytogenes* and *S. enterica* could grow, absence of
*L. monocytogenes* (in all the replicates) was only achieved after 112
days in both acidified smoked (AS) -S and -F hams. Thus, smoking in
combination with acidification provided the best pathogen inhibi-
tion in non-pressurized ham slices. The present results are in
general agreement with literature, where the combined effect of
cold or liquid smoke together with low pH conditions and high salt
concentrations have been observed against *L. monocytogenes* and/
or *Listeria* ham (Smelt, 2002) and meat products (Martin et al., 2010;
Milly, Toledo & Chen, 2008; Montero, Gómez-Estañ & Gómez-
Guillén, 2007). *S. typhimurium* has also been shown to be inhibi-
ted by smoke although its sensitivity is lower than the one
observed for *L. monocytogenes* or other Gram positive bacteria

One of the major problems in the development of NaCl-free
processed products is related to the multifunctional character of
NaCl due to its flavouring and functional contributions and espe-
cially its antimicrobial activity (Sofos, 1983). In dry-cured ham, the
substitution of 50% NaCl by KCl did not affect mesophilic aerobic
and salt tolerant microbiota (Blesa et al., 2008) and partial
replacement of 40% NaCl by KCl in dry fermented sausages main-
tained the microbiological stability of the product (Ibañez et al.,
1995). However, food safety of traditionally dried dry-cured ham
slices, spiked with *L. monocytogenes* and *S. enterica*, was compro-
mised by NaCl substitution with the same ingredients as those
reported in the present study (Stollewerk et al., 2012a). Neverthe-
less, recorded differences could not only be related to the compo-
sition (presence of NaCl, KCl or lactate) but also to the acidification
system (bacterial fermentation or GDL application) and the *dω*
of the product at the time of slicing (finished product in traditional
hams (Stollewerk et al., 2012a) vs. undried or partially dried
product in QDS hams (this study)).

Pressurization at 600 MPa is nowadays industrially applied on
dry-cured meat products (Garriga & Aymerich, 2009), primarily
because of its bactericidal and shelf-life extending effect while
leaving important quality characteristics intact (Knorr, 1993). In the
present study, the application of a HP treatment did not affect
*dω* and did not affect or increased slightly pH, as shown in previous
studies performed on dry fermented sausages (Jofré, Aymerich &
Garriga, 2009; Marcos, Aymerich & Garriga, 2005).

Regarding technological microbiota, pressurization had an im-
mediate lethal effect that was stronger on endogenous LAB than on
starter LAB and GGC+. In pressurized NS-S ham, the only non-
acidified product without any starter, recovery of endogenous
LAB and GGC+ to levels of non-pressurized samples was observed.
Similar behaviour was previously observed in traditionally dried
dry-cured ham (Stollewerk et al., 2012a).

The HP treatment of 600 MPa significantly affected both
*L. monocytogenes* and *S. enterica* counts in all hams, confirming the
listericidial and “anti-salmonella” effect of pressurization, which has
been described before in traditional dry-cured ham (Bover-Cid
et al., 2011, 2012; Hereu, Bover-Cid, Garriga & Aymerich, 2012;
Jofré, Aymerich, Grébol, et al., 2009; Morales, Calzada & Nuñez,
2006; Stollewerk et al., 2012a). Nevertheless, the ham type influ-
enced the efficiency of pressurization and pathogen elimination, in
particular *L. monocytogenes*, was considerably delayed in non-
acidified hams (112 days). The combination of low pH and HP has
been described as an efficient way to inactivate pathogenic mi-
croorganisms in foodstuffs and to inhibit subsequent outgrowth of
sublethally injured cells (Smelt, 1998). However, it was the com-
bination of acidification, smoking and HP that provided the best
protection and achieved immediately after processing absence of
*L. monocytogenes* and after 14 days of storage of slices under
refrigeration absence of *S. enterica*. Similarly, Montero et al. (2007)
observed that smoking and pressurization, together with a high salt
concentration, kept *L. monocytogenes* counts under detection limit
throughout 100 days of storage at 5 °C in cold–smoked dolphinfish.
Comparing the different manufacturings (-S and -F), pathogen in-
hibition was lower in all NaCl-free processed hams, which we
related to the protecting effect of lactate on *L. monocytogenes* dur-
ing pressurization that has been previously reported from tradi-
tionally dried dry-cured ham (Stollewerk et al., 2012a) and cooked
ham (Aymerich, Jofré, Garriga & Hugas, 2005; Jofré et al., 2008).

Hence, the substitution of NaCl and its antimicrobial activity
affected the stability of NaCl-free processed HP treated hams and
produced a significant delay in pathogen elimination.

To sum up, the present study demonstrated for the first time
that in case of a low-level recontamination with *L. monocytogenes*
and *Salmonella* during slicing, QDS dried cured ham produced with
a mixture of potassium lactate, KCl and sugars (-F) was safer than
ham produced with NaCl (-S). The combination of KCl together with
potassium lactate, sugars and GDL in the case of A-F and AS-F
allowed a safe substitution of NaCl in dry-cured ham. The appli-
cation of a HP treatment achieved an additional reduction of
*L. monocytogenes* and *Salmonella* counts and allowed the produc-
tion of an acidified smoked QDS ham (AS-S) that met the EC 2073/
2005 microbiological criteria and the Health Protection Agency
guidelines for *L. monocytogenes* and *Salmonella* (European
Commission, 2005; Health Protection Agency, 2010). Interestingly,
results from the present challenge test also revealed that the
bactericidal effect of pressurization was lower in all NaCl-free
processed hams. This outcome is of great importance for meat
processors and points out that hurdle combinations for the
obtention of safe reformulated and/or modified products must be
fully considered and specifically evaluated.

Acknowledgements

This research was supported by CDTI (Centro para el Desarrollo
Tecnológico Industrial), Ingenio 2010 programme, reference CENIT
2007—2016 (Futural) from Spanish Ministry of Industry, Tourism &
Trade. K. Stollewerk received a doctoral fellowship awarded by the
INIA. The authors thank G. Ferrini for support in experimental
design and Y. Beltran for technical assistance.

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the partial or total substitution of sodium chloride in the elaboration of partially
dehydrated dry cured meat products, the use of such composition and the elabo-
rator process of dry cured meat products partially dehydrated in partial or total
Casademont S.A. and Metalquimia, S.A.
