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Survival of selected foodborne pathogens on dry cured pork loins

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ABSTRACT

The safety of ready-to-eat products such as cured pork loins must be guaranteed by the food industry. In the present study, the efficacy of the dry curing process of pork loins obtained from free-range pigs in the reduction of three of the most important foodborne pathogens is analysed. A total of 28 pork loin segments, with an average weight of 0.57 ± 0.12 kg, were divided into four groups with three being inoculated by immersion with 7 log CFU/ml of either *Salmonella* Typhimurium, *Campylobacter coli* or *Listeria innocua* and the last one inoculated by immersion with sterile medium (control group). The loin segments were treated with a seasoning mixture of curing agents and spices, packed in a synthetic sausage casing and cured for 64 days. Microbiological analysis, pH and water activity (a_w) were assessed at four stages. The values of pH and a_w decreased with curing time as expected. *S.* Typhimurium and *C. coli* dropped significantly (3.28 and 2.14 log units, respectively), but limited reduction of *L. innocua* (0.84 log unit) was observed along the curing process. In our study, three factors were considered critical: the initial concentration of the bacteria, the progressive reduction of pH and the reduction of a_w values. Our results encourage performing periodic analysis at different stages of the manufacturing of dry cured pork loins to ensure the absence of the three evaluated foodborne pathogens.

1. Introduction

The production of safe and healthy products represents one of the main objectives of the food industry worldwide. Food products continue to be responsible for important outbreaks of disease in consumers (Larsen et al. 2014). According to the latest data published by the European Food Safety Authority (EFSA), a total of 4362 foodborne outbreaks were reported in 2015, with *Campylobacter* and *Salmonella* species being responsible for a significant percentage of these cases (EFSA 2016). Another pathogen that has become more important due to its notable and extreme severity is *Listeria monocytogenes* (Álvarez-Fernández et al. 2012; Magalhães et al. 2014). In this scenario, meat products have been identified as significant sources of these foodborne pathogens (Gómez et al. 2014; Holley and Cordeiro 2014).

Animals can be healthy carriers of these pathogens at different organic locations, which can be triggered during stressful situations, such as transport or slaughter procedures. Also, these pathogens can contaminate the meat from the animal's intestinal content or skin which may then be spread by utensils, machinery, water and food handlers and processors (Buncic and Sofos 2012; Choi et al. 2013).

Dry cured pork products obtained from pigs reared under traditional breeding systems, such as the Iberian pig, are highly appreciated by consumers. The characteristic flavour and high quality, in addition to traditional and environmentally and friendly production practices, are important reasons for the growing demand of dry cured hams, shoulders and loins (Ruiz et al., 2002). The manufacture of these products includes the maturation of pork meat with different additives (salts, spices and other ingredients), and the subsequent dehydration and ripening procedures to obtain shelf stable ready-to-eat products (Soto et al. 2008).

Foodborne pathogens such as *Salmonella* spp., *L. monocytogenes* and *Campylobacter jejuni* have been shown to survive some fermentation, maturation and drying procedures necessary to obtain dried and fermented meat products (Lucke 2009; Hong et al. 2016). Furthermore, some of these microorganisms have been detected in cured and fermented dried meats sampled from markets and specialty food shops

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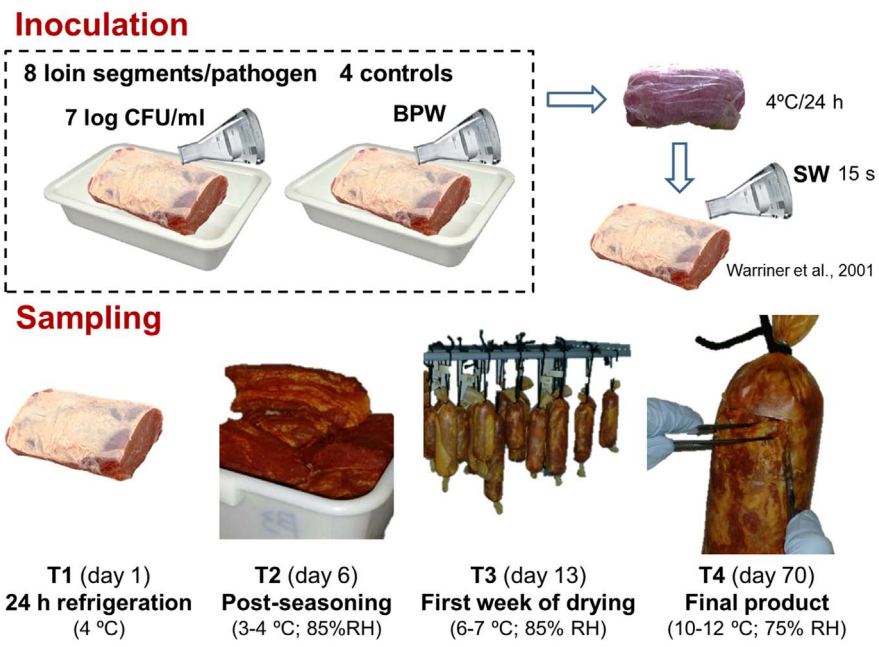

Fig. 1. Inoculation procedure, sampling and processing of the dry cured pork loins along the study. BPW: sterile buffered peptone water. SW: sterile water. RH: relative humidity.

(Gormley et al., 2010). Although previous studies have tested the viability of selected foodborne pathogens during the manufacturing of some dry cured meat products (Reynolds et al. 2001; García-Díez et al. 2016), the efficacy of the manufacturing procedure of dry cured Iberian pork loin to eliminate these microorganisms from contaminated fresh meat has not been previously evaluated.

For that reason, the objective of this work was to evaluate the survival of three pork associated foodborne pathogens, namely *Salmonella* Typhimurium, *Listeria monocytogenes* and *Campylobacter coli*, along the dry curing process of experimentally inoculated free-range Iberian pork loins.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

Monophasic *S.* Typhimurium 1,4,[5],12:i:- DT 193 and *C. coli* strains (one each) used in this study were recovered from pig faeces and identified by specific ISO methodologies (6579:2002 and 10272:2006, respectively). Serotyping of *Salmonella* was performed by means of an agglutination technique using commercially available antisera (Statens Serum Institut, Copenhagen, Denmark) and a phage's panel provided by the International Reference Laboratory of phage typing (Central Public Health Laboratory, London, UK) at the National Centre of Microbiology (Institute of Health Carlos III, Madrid, Spain). A multiplex PCR assay was performed to identify *Campylobacter* to species level (Yamazaki et al. 2008).

Listeria innocua serotype 6a CECT 910 (Spanish Type Culture Collection, Valencia, Spain), originally isolated from a cow brain (Seeliger H. 1979, Wurzburg, Germany), was selected as a surrogate of *L. monocytogenes* to prevent unnecessary exposure to this pathogen during experimental processing (Friedly et al. 2008; Barbiroli et al., 2017).

All the strains were stored at − 80 °C in Brain Heart Infusion (BHI) broth (Oxoid, Madrid, Spain) containing 20% glycerol (Scharlab, Barcelona, Spain) until use.

For inoculum preparation, strains were plated onto Trypticase soy agar (Oxoid) and incubated at 37 °C for 24 h in aerobiosis (*S.* Typhimurium and *L. innocua*), or at 42 °C for 48 h under microaerophilic atmosphere (*C. coli*), using a GENbagMicroaer (BioMérieux, Madrid, Spain). Afterwards, 4 to 5 colonies were isolated and resuspended in 5 ml of BHI medium (Oxoid), and incubated in the same conditions (Merialdi et al. 2015). Then, cultures were counted by using plate counts and absorbance spectrophotometer at a wavelength of 595 nm.

Subsequently, 0.1 ml of this culture were transferred to 9 ml of BHI medium (Oxoid) and incubated at the same conditions to obtain an inoculum of 10^8 CFU/ml of each pathogen that was adjusted to 10^7 CFU/ml by serial dilutions in 0.9% NaCl and 0.1% sterile peptone water solution (Oxoid). Bacterial counts were checked by plating on the same conditions in each assay.

2.2. Dry cured pork loins manufacturing, inoculation procedure and sampling

A total of 14 pork loins (*M. longissimus dorsi*) were obtained from freshly slaughtered Iberian free-range pigs after routine procedures in a commercial slaughterhouse. After butchering, loins were divided into two segments and a total of 28 pork loin segments with an average weight of 0.57 ± 0.12 kg were frozen at − 20 °C until analysis.

For the experiment, loins were allowed to defrost under refrigerated conditions at 4 °C for 2 days. The segments were UV irradiated for 30 min in a laminar flow hood to reduce surface contamination (Keklik et al. 2010). A total of 24 loin segments were inoculated by immersion for 2 min with a concentration of 7 log CFU/ml of each microorganism in 0.9% NaCl and 0.1% sterile peptone water solution (Oxoid) (8 loins/pathogen) to obtain an initial bacterial load of approximately 5 log CFU/ml of each pathogen (Cardoso-Toset et al. 2017). Four additional loin segments were immersed in sterile peptone water solution (Oxoid) and used as controls. After immersion, loins were placed on plastic racks at room temperature (24 °C) for 10 min to allow microbial attachment and stored at 4 °C for 24 h. After chilling, each loin segment was irrigated with water for 15 s to select only superficially attached bacteria (Warriner et al. 2001). Then, a seasoning mixture of curing agents (salt, nitrates and nitrites) and spices (paprika from *Capsicum annuum* and powdered garlic from *Allium sativum*) was added in a ratio of 48 g/kg and macerated for 5 days in plastic vats at 3–4 °C and 85% relative humidity (RH) to allow penetration into the meat (Cardoso-Toset et al. 2017). Following this, the loin segments were packed in a synthetic sausage casing made of collagen and stored at 6–7 °C and 85% RH for a week. Temperature was then increased to 8–10 °C and RH was reduced to 75% for 50 days. Finally, loins were maintained at 10–12 °C