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Survival of *Streptococcus suis*, *Streptococcus dysgalactiae* and *Trueperella pyogenes* in dry-cured Iberian pork shoulders and loins

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ABSTRACT

Dry-cured hams, shoulders and loins of Iberian pigs are highly appreciated in national and international markets. Salting, additive addition and dehydration are the main strategies to produce these ready-to-eat products. Although the dry curing process is known to reduce the load of well-known food borne pathogens, studies evaluating the viability of other microorganisms in contaminated pork have not been performed. In this work, the efficacy of the dry curing process to eliminate three swine pathogens associated with pork carcass condemnation, *Streptococcus suis*, *Streptococcus dysgalactiae* and *Trueperella pyogenes*, was evaluated. Results of this study highlight that the dry curing process is a suitable method to obtain safe ready-to-eat products free of these microorganisms. Although salting of dry-cured shoulders had a moderate bactericidal effect, results of this study suggest that drying and ripening were the most important stages to obtain dry-cured products free of these microorganisms.

1. Introduction

Iberian dry-cured pork products have an important demand of consumers in national and international markets (Gamero-Negrón et al., 2015). The traditional elaboration of these products is based on salting (dry-cured hams and shoulders), addition of a mixture of ingredients and spices (dry-cured loins) and subsequent dehydration and ripening to obtain shelf stable ready-to-eat (RTE) products (Soto et al., 2008; García-Gil et al., 2014).

Dry-cured products are considered to be safe due to several factors such as their low pH and water activity (a_w), their high salt concentration or the addition of nitrites, spices and other ingredients that contribute to their stability (Stollewerk et al., 2012). Previous reports have evaluated the efficacy of the dry curing process to control food-borne pathogens such as *Listeria monocytogenes*, *Salmonella* spp. or *Staphylococcus aureus* (Reynolds et al., 2001; García-Díez et al., 2016). However, the survival of other pathogens that can infect pigs and may contaminate pork meat at slaughterhouse has not been evaluated.

Streptococcus suis, *Streptococcus dysgalactiae* and *Trueperella pyogenes* are swine pathogens that can be isolated from healthy pigs (Lara et al., 2011; Lowe et al., 2011) and that can be also involved in a high rate of suppurative lesions of growing pigs that typically result in partial or total carcass condemnation at slaughterhouse (Martínez et al., 2007; Cardoso-Toset et al., 2015). For that reason, cross contamination of pork along the slaughtering, meat inspection and operational handling can occur (Arai et al., 2015). In fact, previous reports have shown that these microorganisms can be isolated from pork carcasses and raw pork (Martel et al., 2003; Cheung et al., 2008; Blagojevic and Antic, 2014; Brinch Kruse et al., 2015).

S. suis has been recognized as an emerging human pathogen that causes severe infections in pigs and humans in close contact with pigs and pork-derived products, including meningitis, arthritis, septicaemia, pneumonia and endocarditis (Goyette-Desjardins et al., 2014). Although 35 serotypes have been described on the basis of their polysaccharide capsular antigens, *S. suis* serotype 2 is the most prevalent and pathogenic in both species (Arai et al., 2015). The natural habitat of this pathogen is the tonsils and nasal cavities of pigs, but also their genital and digestive tracts (Goyette-Desjardins et al., 2014).

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S. dysgalactiae is another *Streptococcus* species that can be divided into two subspecies based on whole-cell protein profiles and biochemical properties: *S. dysgalactiae* spp. *dysgalactiae*, which includes strains of animal origin and *S. dysgalactiae* spp. *equisimilis* which includes strains of both animal and human origin (Martínez et al., 2007; Silva et al., 2015). This subspecies has increasingly been recognized as etiological agent of several human invasive infections worldwide, including pharyngitis, septic arthritis, pneumonia, endocarditis, meningitis, streptococcal toxic shock-like syndrome, cellulitis and necrotizing fasciitis (Takahashi et al., 2011; Silva et al., 2015).

Finally, *T. pyogenes* (formerly *Arcanobacterium pyogenes*) is a well-recognized coryneform bacterium related to miscellaneous opportunistic pyogenic infections among cattle, sheep, pigs and goats, species in which this microorganism is usually found on the skin, oropharynx, upper respiratory, urogenital and gastrointestinal tracts (Ribeiro et al., 2015). In contrast, *T. pyogenes* has been sporadically associated with human infections, including endocarditis, pneumonia and sepsis (Hermida Ameijerias et al., 2004; Levy et al., 2009).

In this study the survival of *S. suis, S. dysgalactiae* and *T. pyogenes* along the dry curing process of experimentally inoculated pork shoulders and loins is evaluated. Results of this study can help to better understand the efficacy of this procedure to obtain safe RTE products free of these potential biological hazards.

2. Materials and methods

2.1. Preparation of the experimental dry-cured pork shoulders

Dry-cured shoulders were selected instead of dry-cured hams due to their shorter ripening period (14–18 months vs more than 24 months), which makes it possible to draw conclusions about dry-cured products in less time (Gamero-Negrón et al., 2015). Seventeen homogeneous sized pork forequarters with an average weight of 4 kg and in a pH range from 5.5 to 6.0 were obtained from freshly slaughtered Iberian free-range pigs at a local slaughterhouse. After collection, shoulders were frozen to −20 °C until analysis. Before inoculation, pieces were thawed at refrigeration temperature (4 °C) for 4 days.

After inoculation (described in 2.2.2), pork shoulders were salted with a traditional 100% NaCl formulation on plastic vats in a cold room held at 3–4 °C and 85% relative humidity (RH) during 5 days (1.25 day/kg) following routine procedures (García-Gil et al., 2014). A small portion of salt was mixed with nitrificant salts and applied by rubbing and kneading to each shoulder as curing agents (Armenteros et al., 2012). After salting, pieces were pressure washed with warm water to remove the remaining salt from their surfaces and held at 3–4 °C and 85% RH for 40 days (post-salting phase). Temperature was thereafter increased from 4 to 24 °C at 0.5 °C/day during 40 days and RH was progressively reduced to 65% (drying stage). The final ripening was carried out during 14 months at room temperature (14–24 °C) (Table 1).

2.2. Preparation of the experimental dry-cured pork loins

Ten pork loins (*Longissimus dorsi*) from freshly slaughtered Iberian free-range pigs (two loins/pig) were obtained after routine slaughter procedures at a local slaughterhouse and stored at −20 °C until analysis. Before inoculation, loins were thawed at refrigeration temperature (4 °C) for 2 days and divided into three pieces. A total of 28 pieces with an average weight of 0.53 kg ± 0.06 kg and a pH value from 5.70 to 6.15 were obtained.

After inoculation (described in 2.2.3), pork loins were rubbed with a seasoning mixture of curing agents (salt, nitrates and nitrites), spices and condiments including paprika (*Capsicum annuum*) and powdered garlic (*Allium sativum*) as detailed by Soto et al. (2008) in a relation of 48 g/kg and macerated at 4 °C for 5 days to allow the penetration of the seasoning mixture into the meat. Afterwards, every loin was stuffed into a collagen case (90 mm in diameter) and ripened. Products were kept at 6–7 °C and 85% RH for a week. Then temperature was increased to 8–10 °C and RH was reduced to 75% during 50 days. Finally, loins were maintained to 10–12 °C and 75% RH until the end of the ripening process (77 days) (Table 1).

2.3. Bacterial strains and inoculum preparation

T. pyogenes (C48ABS), *S. suis* serotype 2 (S17G) and *S. dysgalactiae* spp. *equisimilis* (S32P) strains used in this study were previously isolated from pig carcasses condemned at slaughterhouse and identified by biochemical and molecular techniques (Blume et al., 2009; Cardoso-Toset et al., 2015). Bacteria were stored at −80 °C in Brain Heart Infusion (BHI) broth (Oxoid, UK) containing 20% glycerol (Sharlau, Spain) until use.

Strains were plated on Columbia blood agar base supplemented with 5% sterile defibrinated sheep blood (Oxoid, UK) and incubated for 24 h at 37 °C under a 5% CO_2 enrichment atmosphere. Four colonies of *T. pyogenes* were transferred to 30 ml of BHI broth supplemented with 10% bovine serum albumin (Sigma Aldrich, Spain) and incubated overnight to obtain and OD_{600} of 0.6 (5×10^8 CFU/ml). When streptococci were evaluated, 4 colonies were transferred to 30 ml of Todd-Hewitt broth medium (THB, Oxoid, UK) and incubated overnight at 37 °C under aerobic conditions (pre-inoculum). Then, 0.1 ml were transferred to 9 ml of THB and incubated under the same conditions until the OD_{600} was 0.5 for *S. suis* and 0.4 for *S. dysgalactiae* (5×10^8 UFC/ml each). Each inoculum was subjected to ten-fold serial dilutions in a 0.9% NaCl and 0.1% sterile peptone water solution to obtain a bacterial suspension of 5–6 log CFU/ml as final inoculum. Bacterial counts were checked by plating on Columbia agar (Oxoid, UK) in each assay.

2.4. Inoculation of pork shoulders

Fifteen pork shoulders were inoculated with the evaluated microorganisms (five shoulders/pathogen) and two shoulders were inoculated with sterile PBS and kept as controls. To evaluate the efficacy of the dry-curing process to eliminate the presence of these pathogens in deeper structures of the pork shoulders (e.g. after lympho-hematic spread), the microorganisms were injected into the meat. Five areas of 5×5 cm^2 were systematically marked on the meat of the inner and external side of each piece using a sterile template and sterile pins (Reynolds et al., 2001). Then, for every pathogen 1 ml of a 5 log CFU/ml inoculum was injected in each area using sterile syringes and needles (BD, Spain) and dipping approximately 2 cm into the meat. Later on, pieces were kept at room temperature (24 °C) for 10 min to allow the attachment and absorption of the inoculum. Pieces were maintained at 4 °C for 24 h until analysis.

2.5. Inoculation of pork loins

For each microorganism, eight pieces were inoculated by immersion for 2 min in a 6 log CFU/ml peptone water solution. Four pieces were immersed in a sterile peptone water solution 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 on cling film. After chilling for 24 h, each loin was irrigated with water for 15 s to select superficially located attached bacteria according to Warriner et al. (2001).