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Behavior of *Salmonella* spp. and *Listeria monocytogenes* throughout the manufacture and shelf-life of dry-cured formed ham

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ABSTRACT

In contrast to traditional raw ham produced from a whole muscle or from a combination of different parts, dry-cured formed ham consists of many small meat pieces and thus has a high surface area. As a result, foodborne pathogens originating from contamination could grow if they would be transferred into the interior of the formed product. The aim of this study was to evaluate the behavior of *Salmonella* spp. and *Listeria monocytogenes* during the manufacture and shelf-life of dry-cured formed ham. The potential origins of pathogens could be from contaminated raw meat, added ingredients, processing equipment, and post-processing. The results of this study showed that the combination of the intrinsic (pH, water activity, and microflora) and the extrinsic properties (fermentation and drying conditions) prevented the growth and lead to reduction of the foodborne pathogens during manufacturing of dry-cured formed ham as well as during the shelf-life of 35 days at 4 °C.

1. Introduction

According to the European Food Safety Authority and European Centre for Disease Prevention and Control (2014), the number of confirmed human cases of salmonellosis and listeriosis in the European Union in 2012 was 91034 and 1642, respectively. These foodborne pathogens in foodstuffs were mainly detected in meat and Ready-to-Eat (RTE) products of meat origin. The Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for RTE foods have specified that *Salmonella* spp. must be absent in 25 g and *Listeria monocytogenes* must not exceed the limit of 100 cfu/g at the end of the shelf-life. According to the Codex Alimentarius, a RTE product is “any food which is normally eaten in its raw state or any food handled, processed, mixed, cooked, or otherwise prepared into a form which is normally eaten without further listericidal steps” (CAC, 2007). Within the group of meat-based RTE products in the last years dry-cured formed ham has gained importance as an alternative to traditionally raw ham. Dry-cured formed hams are a new category of aggregated (or re-assembled) raw ham products, where relatively small pieces of meat with an edge length of 2–10 cm can be used as raw material (Beneke et al., 2011; Islam, 2011; Kaufmann, Koppel, & Widmer, 2012). For this purpose cold set binding technologies like enzymatic crosslinking using transglutaminase may be used, which provide economic advantages and advantages in meat processing (Boles, 2011).

In contrast to traditional raw ham produced from a whole muscle or from a combination of different parts, dry-cured formed ham consisting of multiple small meat pieces has a high surface area in the initial stages of processing. Consequently, dry-cured formed ham may possess an increased risk of the growth of foodborne pathogens transferred into the interior of the formed product during manufacturing. Foodborne pathogens such as *Salmonella* spp. and *L. monocytogenes* may originate from contaminated raw meat, added ingredients, processing equipment, or as a result of contamination during post-processing steps, for example during slicing or packaging.

The safety of a marketable food product has to be ensured. For that purpose, challenge tests have been introduced as very important means to investigate the behavior of microorganisms in new or modified food products, in new process developments or modifications, or in new food recipes (EC, 2008). To the best of our

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knowledge, there is no study reporting on the behavior of the foodborne pathogens *Salmonella* spp. and *L. monocytogenes* in dry-cured formed ham made from small meat pieces. Therefore, the aim of this research was to investigate the survival capability of the foodborne pathogens *Salmonella* spp. and *L. monocytogenes* during the production of dry-cured formed ham, and on the other hand, to evaluate the growth potential of these pathogens on sliced and vacuum packed dry-cured formed ham. In non-contaminated dry-cured formed ham additional microbiological assessments and sensory evaluation were carried out to confirm the quality during the intended shelf-life.

2. Materials and methods

2.1. Experimental design

Two different experiments were performed. In the first experiment – the manufacturing trial – the raw meat material was artificially inoculated with *Salmonella* spp. or *L. monocytogenes* to investigate the behavior of these pathogens throughout the manufacturing process including 35 days of ripening simulating contamination of raw meat, added ingredients and processing equipment. In the second experiment – the storage trial – slices of dry-cured formed ham were artificially inoculated with the pathogens to investigate their behavior throughout the shelf-life of 35 days in order to simulate contamination of the final product after manufacturing. *Salmonella* spp. and *L. monocytogenes* were measured in both trials on day 0, 1, 3, 7, 14, 21, 28, 35. Inoculation using a sterile physiological saline solution was performed to prepare control samples in both trials. All samples were prepared with three independent replicates resulting in a total number of 72 samples per trial. For microbial analyses, three samples were analyzed in duplicate on each measuring day. Experiments were performed three times independently resulting in 18 data points per measuring day.

2.2. Bacterial strains and inoculum preparation

Five different strains of *Salmonella* spp. and *L. monocytogenes* were used for inoculation. The strains of *Salmonella* used were *S.* Infantis S506 isolated from Mettwurst (a traditional smoked and short-ripened German sausage), *S.* Enteritidis S554 isolated from a patient, *S.* Derby S587 isolated from fresh pork meat, *S.* Goldcoast S628 isolated from mechanically desinewed pork meat and *S.* Typhimurium S702 (ATCC 14028). The *L. monocytogenes* strains used were Li265 (serotype 1/2a) isolated from minced pork meat, Li23 (serotype 4b) isolated from Vesperwurst (a traditional smoked and short ripened German sausage with onion), Li146 (serotype 4d) isolated from batching stations, Li142 (serotype 1/2c) isolated from dry-cured ham manufacturing plant and Li111 (serotype 1/2b, SLCC 8681). All strains are stored as a lyophilisate at 4 °C in milk powder (10 wt %); they belong to the collection of bacterial strains of the Max Rubner-Institut – Federal Research Institute of Nutrition and Food (Kulmbach, Germany). Strains were grown separately overnight at 37 °C in Brain Heart Infusion Broth (BHI Broth, SIFIN Diagnostics GmbH, Germany). Individual isolates were then stored at −70 °C in Microbank vials (Pro-Lab Diagnostics, United Kingdom). For each experiment, one bead from a culture of each strain was added to BHI Broth and incubated at 37 °C for 24 h. A mixture of *Salmonella* spp. or *L. monocytogenes* was prepared by adding 2 ml of each strain to a sterile tube and vortexing. The concentration of the mixture suspension was determined with a THOMA-counting chamber (Jena Fein-Optik, Germany) under a light microscope (Leica Diaplan, Leica Mikrosysteme Vertrieb GmbH, Germany). The mixture suspensions were diluted to a final concentration of approximately 1×10^4 cfu/g in dry-cured formed ham meat mixture in case of manufacture trial and 3×10^3 cfu/g in dry-cured formed ham in the storage trial. A lower concentration was chosen in the storage experiment since it was presumed that during manufacturing the number of cells will decrease and the contamination level would be lower in the final product.

2.3. Preparation of microbial transglutaminase (TG)

The TG formulation (Microbial transglutaminase: Activa PB, Ajinomoto Foods GmbH, Hamburg, Germany) used in a freeze dried form contains calcium chloride, pork protein, maltodextrin, silicon dioxide, transglutaminase with an activity of 63 units/g and vegetable oil. According to the instructions of the supplier, a slurry was made as follows: 0.8 wt % (relative to meat) of TG formulation was added to ice water (ratio 1:5) and mixed (Ultra-Turrax T 18 basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) until a homogeneous paste was obtained.

2.4. Preparation of dry-cured formed ham

The manufacturing steps are displayed in Fig. 1. Fresh pork meat (*M. longissimus dorsi*) was purchased 48 h post mortem from a local slaughterhouse (Schiller Fleisch GmbH, Hof, Germany). All visible fat, tendons and connective tissue were trimmed off. The meat was then diced to cubes with an edge length of approximately 2 cm. Meat cubes were then tumbled (Frig-o-Vac System Type 180/14, BTE Maschinenbau GmbH, Murg, Germany) at 2 °C and 7 rpm for 18 h under a vacuum of 20 kPa to extract salt soluble proteins. The following ingredients were added before the tumbling process: 3% nitrite curing salt containing 99.5% sodium chloride and 0.5% sodium nitrite (Südsalz GmbH, Heilbronn, Germany), 0.5% saccharose (Merck KGaA, Darmstadt, Germany), 0.5% D(+)-glucose monohydrate (Merck KGaA, Darmstadt, Germany), and 0.05% sodium L(+)-ascorbate (Merck KGaA, Darmstadt, Germany). All percentage values are related to the weight of the meat (wt %). After the tumbling process, for the manufacturing trial, the meat-mixture was divided into three parts. Two parts part were inoculated with the pathogen strains reported above (mixture suspensions of *salmonella* spp or *L. monocytogenes*), and hand-mixed for 5 min. Another part of the tumbled meat was inoculated with sterile physiological saline solution and used as control to determine the general microbiological and physicochemical properties of the samples. The binding agent TG (TG-slurry reported above) was added to the meat-mixture and hand-mixed for another 5 min. Afterwards the meat-mixture was filled manually into Nalo cellulose casings, calibre 90 mm (Kalle GmbH, Wiesbaden, Germany). The weight of each of the formed sample was approximately 1 kg. Samples were hung at 2 °C and at 75% relative humidity (rH) in a refrigeration room for 7 days (salting period), thereafter the samples were transferred into a chamber (Allround System Rondair, MC 3.2, Maurer AG, Reichenau, Germany), where temperature of 15 °C and rH of 85% were maintained until the end of the experiment (35 days).

For the second experiment (storage trial) dedicated to simulate contamination after manufacturing non-inoculated dry-cured formed hams were prepared as described above. After 21 days of manufacture, the hams were sliced into 1 mm thickness. Out of two slices (total mass 25 g), one slice was surface inoculated by spreading 100 µl of diluted mixture of *Salmonella* spp. or *L. monocytogenes* until completely absorbed, and one non-inoculated slice was placed on top of the inoculated slice. The pair of slices was packed in PA/PE plastic bags (oxygen permeability $\leq$ 60 cm^3/m^2/24 h/bar (23 °C, 50% rH), water vapor permeability $\leq$ 1.7 g/m^2 24 h/bar (23 °C, 85% rH)) (Siegelrand-