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Lactic acid bacteria in cooked hams – Sources of contamination and chances of survival in the product

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ABSTRACT

The aim of this study is microbiological analysis of individual technological operations during the industrial production of cooked hams, focusing on lactic acid bacteria (LAB). Samples were during the course of the entire cooked ham production cycle in May–June (Experiment I) and November–December (Experiment II). A total of 215 samples were taken and subsequently tested. The difference in the occurrence of LAB in meat before thermal processing resulted from the initial level of contamination of the raw material. A reduction to the number of LAB from 4.0 to 5.0 log CFU/g of meat to a value of practically zero occurred during the thermal processing. The LAB population increased during storage of the finished products. A level of 7.0 log CFU/g was reached in slices of ham in the modified atmosphere after three (Experiment I) or two (Experiment II) weeks of storage, respectively. LAB of the genera *Leuconostoc* (*Leuc. carnosum*, *Leuc. mesenteroides* and *Leuc. gelidum*) occurred most frequently in samples of cooked ham after thermal processing. These species were also isolated from the production environment. *Lactobacillus sakei*, *Lbc. curvatus* and *Weissella viridescens* were other species of LAB that were isolated from samples after thermal processing.

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1. Introduction

Cooked hams are popular meat products in many countries (Tomović et al., 2013). Customers appreciate this type of product both for its sensory properties and, first and foremost, for its high protein content and low percentage of fat. Cooked hams are thermally processed meat products. Thermal processing plays an important role in the selection of bacteria that may find their way into the product along with the raw material (meat) and additives used or from the production environment (Comi & Iacumin, 2012). Thermal processing is not, however, always effective, particularly in respect of thermotolerant vegetative bacteria, and has no effect on the secondary contamination that may occur in product handling after heating, particularly during slicing and subsequent packaging (Vasilopoulos et al., 2010). Storage and distribution at refrigerated temperatures and packing in a modified atmosphere or vacuum are used to protect thermally processed meat products and to ensure their guaranteed shelf life (Pothakos, Samapundo, & Devlieghere, 2012). The shelf life of sliced cooked hams packed in a modified atmosphere or vacuum is, nevertheless, limited to between three and six weeks (Leroy, Vasilopoulos, Van Hemelryck, Falony, & De Vuyst, 2009).

Lactic acid bacteria are the main bacterial group associated with the spoilage of thermally processed meat products, including cooked hams (Samelis, Kakouri, & Rementzis, 2000; Vermeiren, Devlieghere, De Graef, & Debevere, 2005). Their growth is favoured by a combination of microaerophilic conditions in the product, the presence of sodium chloride and sodium nitrite, and a reduced water activity value (Audenaert et al., 2010). These factors suppress the growth of other microbial groups thus providing a selective advantage to the LAB. It is still not, however, entirely clear to what degree the bacteria present in meat products come from

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the meat or from the environment, with contamination occurring subsequently during handling (Vasilopoulos et al., 2010). The literature contains studies on the development of contaminating LAB in cooked hams during the production process and subsequent storage (Leroy et al., 2009; Matagaras, Skandamis, Nychas, & Drosinos, 2007; Vermeiren et al., 2005; Vasilopoulos et al., 2010). None of these studies, however, provide information on the level of contamination of meat before the production process begins, i.e. before the injection of brine, and the authors of these studies have also not conducted microbiological tests of the environment in which these hams were produced.

The aim of this study is, for this reason, microbiological analysis of individual technological operations during the industrial production of cooked hams, focusing on LAB. The experiment takes in the input raw material at the initial stage of production, i.e. at the slaughterhouse, and also focuses on key sites in production plants, i.e. cutting room, injecting and tumbling, and final slicing and packaging.

2. Materials and methods

2.1. Materials

2.1.1. Meat and cooked hams

Sampling was performed at the plant of an industrial meat producer with an average daily production of around thirty tons of cooked meat products and also operating a slaughterhouse with a capacity of approximately three hundred pigs and eight cattle a day. Samples were taken for microbiological analysis during the course of the entire cooked ham production cycle in May–June 2013 (Experiment I) and November–December 2013 (Experiment II).

The cooked ham was made of pork leg (silverside). The meat was injected with brine in a Fomaco injector (Fomaco A/S, Køge, Denmark). The quantity of brine was 18% of the input weight of the meat. Brine composition: diphosphates, triphosphates, saccharose, sodium ascorbate and processed Eucheuma seaweed. The NaCl content (nitrite curing salt) in the finished product was 2%. Injection was followed by tumbling (Inject Star, Hagenbrunn bei Wien, Austria) for a period of 12 h (50 min tumbling, 10 min rest, 6 rpm) at 6 °C in a vacuum. The next day, the tumbled pieces of meat were manually filled in plastic bags of a size of 800 × 600 mm (PA/EVOH/PA/PE with oxygen transmission rate [OTR] < 5 cm^3/m^2/24 h/24 °C). The filled bags of a weight of 12 kg were vacuum-packed and placed in moulds in which they were thermally processed to a core temperature of 70 °C for 10 min. This was followed by cooling to 4 °C. Slicing was performed three days after thermal processing in a MegaSlicer automatic slicer (GEA Food Solutions, Kempten, Germany) with subsequent packaging of the slices in a Multivac thermoforming packing machine (Wolfertschwenden, Germany) in a modified atmosphere (N_2: CO_2/70: 30) using a film with an OTR of 5 cm^3/m^2/24 h/24 °C (Biaxer 65 XX, Wipak Prague, Czech Republic). The packages, weighing 100 g, were stored at 2 ± 2 °C for the duration of the experiment.

In Experiment I, 2 × 10 samples were taken from pig carcasses in 45 min *post mortem* before cooling (10 by a destructive technique and 10 by a non-destructive technique from the same carcasses) on Day 0. This was followed by the traditional chilling of the carcasses at an air temperature of 2 °C. The following day (Day 1), the chilled carcasses (core temperature 5 °C) were cut up and 10 swab samples were taken from the surface of the silverside of pork leg. Brine was prepared on Day 2 and the meat was injected in an injector. Seven swab samples were taken from the surface of the meat prepared in this way following injection. The injected meat was tumbled on Day 3, after which another 7 swab samples were taken. The meat was then placed in a plastic bag, sealed in a vacuum, and thermally processed in moulds to a core temperature of 70 °C for 10 min. This was followed by cooling at 2 °C, after which the finished cooked hams were taken out of the moulds and stored at 2 °C. Four samples of cooked hams were taken before slicing on Day 9, after which the hams were sliced and packaged in a modified atmosphere. Slices of ham in the modified atmosphere were analysed immediately after packing (Week 0) and then again in Weeks 1, 2, 3, 4, 5 and 8. A total of 116 samples of meat and/or samples of cooked hams and 4 samples of brine were tested in Experiment I.

In Experiment II, samples were taken from 20 pork silversides (10 of Czech origin from the company slaughterhouse and 10 of German origin – imported). Six samples were taken from these pork legs the following day after tumbling. This process was performed on the same machine and under the same conditions as in Experiment I. Cooked ham was prepared from this raw material. Four samples of ham (thermally processed and cooled as in Experiment I) were taken on Day 7 after Experiment II began. This was followed by slicing and packaging in a modified atmosphere. Six samples (of 100 g) of sliced and packaged hams were taken immediately after packing and again in Weeks 1, 2 and 3. A total of 58 samples of meat and/or cooked hams were tested in Experiment II.

A total of 174 samples of meat and cooked hams and 4 samples of brine were tested in Experiments I and II.

2.1.2. The environment

Swab samples from the environment were taken from cutting room, the whole-muscle meat product production area and the packing area. Experiment I – samples were taken from the surface of the hands of cutting room workers (3 ×), the surface of a worktable (2 ×), plastic boxes (2 ×), hooks for meat hanging (2 ×) and cutting room staff aprons (2 ×) on Day 1. Swab samples were taken from the surface of a worktable (1 ×), the hands of an employee (1 ×), plastic box (1 ×) and an injector (2 ×) on Day 2. Samples were taken from the inner surfaces of tumblers (5 ×) on Day 3. Swabs were taken from the work surfaces of the slicing machine (6 ×), a handling table (1 ×), scales (1 ×) and a packing machine (2 ×) on Day 9. A sample of fragments of sliced products that had accumulated at one spot behind the knife of the slicing machine during a working shift was also taken. Experiment II – samples were taken from the work surfaces of the slicing machine (3 ×) and samples of accumulated fragments (2 ×). A total of 37 samples were taken from the environment for testing in Experiments I and II.

Samples from the environment and the surface of raw materials were taken with sterile cotton swabs from an area of around 100 cm^2. The swabs were placed in test tubes with sterile MRS broth (Oxoid, Basingstoke, UK) immediately after collection and transported at a temperature of 4 ± 2 °C to the laboratory for microbiological testing.

A total of 215 samples were taken and subsequently tested in the two experiments.

2.2. Methods

2.2.1. Detection and enumeration of microorganisms

Basic processing of the samples was carried out in accordance with the standards ISO 7218 (2008) and ISO 6887-1 (1999). Cooked ham samples weighing 500 g were taken for the purpose of analysis. Twenty-five grams of sample was taken in a sterile manner from at least three parts of the ham and homogenised in 225 mL of sterile MRS broth (Oxoid, Basingstoke, UK) in a BagMixer® 400 stomacher (Interscience, St Nom la Bretêche, France).

Enumeration of LAB was performed on MRS agar (Oxoid, Basingstoke, UK) (30 °C/72 h). MRS broth (15 and 30 °C/24 h in

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