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Decontamination of incoming beef trimmings with hot lactic acid solution to improve microbial safety of resulting dry fermented sausages – A pilot study

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ABSTRACT

In the study, beef trimmings intended for sausage production were subjected to different decontamination treatments based on lactic acid-hot water combination, with aim to eliminate or reduce foodborne pathogens *Escherichia coli* O157, *Salmonella* Typhimurium and *Listeria monocytogenes* in resultant dry, fermented sausages. In finished sausages, produced from untreated trimmings, "natural" reductions of inoculated *E. coli* O157 and *Salmonella* Typhimurium were on average 1.9 logs, *L. monocytogenes* count remained unchanged, and no detectable concentrations of biogenic amines were found. The same type of sausages were also produced by using beef trimmings which was pathogen-inoculated and then decontaminated by: hot, 4% lactic acid in water solution (90 °C for 10 s; treatment HLA1); or hot, 4% lactic acid in water solution (85 °C for 20 s; treatment HLA2), or hot, 4% lactic acid in water solution (80 °C for 30 s; treatment HLA3). The use of HLA-decontaminated beef trimmings resulted in total *E. coli* O157 reductions of at least 3.9 logs and in total *Salmonella* Typhimurium reductions of at least 3.6 logs, whilst biogenic amines were not detected in finished sausages. The overall sensorial acceptability of finished sausages produced with HLA-decontaminated beef trimmings was somewhat diminished. Further work is required to optimise the HLA-based incoming beef treatments.

1. Introduction

The production process of fermented sausages is conducted at relatively high temperatures, which vary with sausage types roughly between 20 °C and 40 °C, and involves two distinct stages. During the first 2–3 days, the fermentation takes place, which is associated with production of lactic acid (pH drop) by fast-multiplying, microaerophilic lactic acid bacteria (LAB) "replacing" the initial aerobic, psychrotrophic meat microbiota. Subsequently, the maturation, commonly including drying, takes place for time periods varying with sausage type (whether spreadable, semidry or dry) between a few days to a few weeks. The microbial safety and storage stability (shelf-life) of fermented sausages depend on combined effects of multiple antimicrobial factors acting in the sausage matrix: acidity (low pH), low water activity (a_w) due to added salt and drying, nitrites, and LAB due to their competitiveness and production of inhibitory compounds such as bacteriocins (Lücke, 2000). Generally, in raw, fermented sausages produced under proper and controlled conditions, microbial foodborne pathogens can survive but their counts are reduced and they usually do not multiply. Microbial safety concerns associated with fermented sausages relate to: a) primarily, bacterial foodborne pathogens originating from raw materials used for sausage production (i.e. incoming meat); and b) to a lesser extent, toxic biogenic amines (BA) such as histamine and tyramine produced by background microbiota.

A number of outbreaks of toxicoinfections by verocytotoxigenic *Escherichia coli* (VTEC) associated with consumption of fermented sausages (mainly containing beef, but also lamb) have been reported in different countries, as reviewed by Holck et al. (2011).

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Also, consumption of fermented sausages has been associated or epidemiologically linked with other foodborne illnesses caused by *Staphylococcus aureus* (Bacus, 1986), *Salmonella* (Sauer, Majkowski, Green, & Eckel, 1997) or *Listeria monocytogenes* (WHO/FAO, 2004). Literature data clearly indicate that fermented sausages pose an increased potential risk with respect to high content of the main, toxic biogenic amines (BA) histamine and tyramine, and they also can contain other biogenic amines (e.g. putrescine and cadaverine) which are less toxic themselves but can potentiate the toxic effects of the former two (Ruiz-Campilas & Jimenez-Comlenero, 2004). BA accumulation is usually related to the decarboxylase activity of contaminant bacteria (e.g. enterobacteria, pseudomonads), but technological bacteria such as LAB may also contribute significantly to aminogenesis (Bover-Cid, Hernández-Jover, Miguélez-Arrizado, & Vidal-Carou, 2003; Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2001). Hence, the BA accumulation in fermented sausages can be reduced by using non-BA-forming LAB starter cultures (Bover-Cid et al., 2001). Although no cases of histamine or tyramine intoxications have implicated fermented sausages as vehicles todate, the products may occasionally contain these BA at levels generally considered as toxic (EFSA, 2011), and the lack of the published intoxications may be due to underreporting.

In respect to the pathogen of particular concern in beef/lamb fermented sausages, VTEC, it is known that it survives the standard fermented sausage production process and a 5-log reduction, required by regulation in the USA (Reed, 1995) but not in the EU, cannot be consistently achieved solely through manipulations of product pH, salt/nitrites and a_w levels (Glass, Loeffelholz, Ford, & Doyle, 1992). Consequently, various heat treatment regimes, applied to fermented sausages either at the post-fermentation or post-maturation stages of production process and aimed at VTEC-reduction, have been evaluated, as reviewed by Holck et al. (2011). The heat treatment of fermented sausages is often used in practice (and accepted by consumers) in the USA, but is used very rarely, if at all, in Europe.

An alternative, more preventative approach to controlling microbial hazards in beef fermented sausages could be decontamination of incoming raw beef; however, no such studies have been published to date. Hence, the main objective of the present study was to evaluate the potential of decontaminating the incoming beef trimmings, based on combined effects of heat and lactic acid, to control the main microbial pathogens and BA in fermented sausages produced from these trimmings.

2. Materials and methods

2.1. Inoculation of incoming raw beef

Strains of pathogens *E. coli* O157 (four strains: ATCC 35150 and three strains from our own collection, isolated from bovine intestines), *S.* Typhimurium (two strains: ATCC 14028 and ATCC 13311) and *L. monocytogenes* (five strains: ATCC 19112, ATCC 19114, ATCC 19115 and two strains from our own collection, isolated from beef sausage and from smoked rib eye steak). Multiple strains per pathogen were used to accommodate for well-known between-strain diversity in respect to both growth potential and inactivation dynamics. Each strain was individually grown in mEC + Novobiocin selective enrichment broth (Merck, Germany), Rappaport Vassiliadis Broth with soya broth (Merck) and L-PALCAM broth (Merck) at 37 °C, 41.5 °C and 30 °C, respectively for 12 h. Appropriate volumes of each strain were added to distilled water so to obtain multi-pathogen, multi-strain suspension containing approximately 4 logs CFU of each pathogen per ml of water. Beef trimmings (pieces roughly 300 g each, chilled at approximately 2 °C) were surface inoculated by submersion for 10 s in the suspension of pathogens, and drained for 10–15 min.

2.2. Decontamination of inoculated beef trimmings

Inoculated beef trimmings were divided into groups, and each group subjected to one of the following surface decontamination treatments:

(a) hot lactic acid treatment 1 (HLA1): beef trimmings were submerged in hot 4% lactic acid water solution (90 °C) for 10 s;
(b) hot lactic acid treatment 2 (HLA2): beef trimmings were submerged in hot 4% lactic acid water solution (85 °C) for 20 s; and
(c) hot lactic acid treatment 3 (HLA3): beef trimmings were submerged in hot 4% lactic acid in water solution (80 °C) for 30 s.

Fatty tissue used for sausage production was subjected neither to inoculation nor to decontamination treatments described above, because hot water melts the fat and makes the treated fatty tissue unsuitable for production of fermented sausages.

2.3. Preparation of raw, fermented, dried beef sausages ("Sudzuk" type)

Four groups of sausages were prepared: a) by using inoculated but not decontaminated beef trimmings (control for treatments); b) with inoculated and HLA1-decontaminated beef; c) with inoculated and HLA2-decontaminated beef; and d) with inoculated and HLA3-decontaminated beef. In addition, within each of groups b), c) and d), some sausages were not inoculated with pathogens, but were subjected to corresponding decontamination treatment (destined for sensory panel examination, see below). Apart from differences in the status of incoming beef, as indicated above, the formulation and production process for all the sausages were the identical. Beef trimmings from leg (45%), beef trimmings from shoulder (45%) and beef fat (10%) were chopped in meat mincer (La Minerva A/E22, Italy) and transferred to kneader mixer (Mainca RM20R, Spain). During meat mixing, 2% of commercially-supplied salt for curing (containing sodium nitrite), 0.25% dextrose and 0.8% spices (powdered garlic, black pepper and paprika) were added to the batter in the kneader mixer. Then, a starter culture comprising LAB strains *Lactobacillus sakei* CTC 41, *L. sakei* CTC 287, *L. sakei* CTC 6469 and *L. sakei* CTC 6626 (all identified as not producing biogenic amines, obtained from Dr. Sara Bover-Cid, Institute for Food and Agricultural Research and Technology-IRTA, Spain) was added at level 4×10^5 of each strain per gram of sausage batter. Subsequently, the sausage batter was stuffed into collagen casings (Koko ø35, Viscofan, Serbia) and sausages were placed in a controlled-environment chamber (Stagionello STG 100 MTF, Italy) and subjected to a fermentation and maturation/drying process for 20 days in total, under the following temperature/relative air humidity conditions: 1st day – 20 °C/92%; 2nd day – 19 °C/86%; 3rd day – 18 °C/80%; 4th day – 17 °C/72%; 5th to 20th day – 16 °C/65%). There were three sausages sampling points during the process: at day 0 (immediately after stuffing into casings), at day 3 (on completion of fermentation) and at day 20 (finished product).

2.4. Sampling and sample homogenates preparation

Sausage samples (100 g) were placed in homogeniser bags with filters (6 × 9″, Nasco Whirl-Pak, USA), 100 ml of maximum recovery diluent (MRD; Oxoid, UK) was added, and each bag was repeatedly