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Use of ionizing radiation doses of 2 and 4 kGy to control *Listeria* spp. and *Escherichia coli* O157:H7 on frozen meat trimmings used for dry fermented sausage production

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Abstract

This study evaluated survival of *Listeria* spp. (four-strain mixture of *Listeria innocua* plus a non-virulent *Listeria monocytogenes* strain) and *Escherichia coli* O157:H7 strain ATCC 43888 during fermentation and ripening of Greek dry sausages formulated from meat and pork fat trimmings previously inoculated with ca. 6 log cfu g^{-1} of the target bacteria and then irradiated in frozen (−25 °C) blocks at doses of 0 (control), 2 or 4 kGy. Irradiation of the trimmings at 2 kGy reduced initial contamination of the sausage batter with *Listeria* and *E. coli* O157:H7 by 1.3 and 2.0 log cfu g^{-1}, respectively, while the corresponding reductions at 4 kGy were 2.4 and 5.5 log cfu g^{-1}, respectively. In fact, *E. coli* O157:H7 was eliminated by 4 kGy at formulation (day 0) as compared to 7 and 21 days of ripening in samples treated at 2 and 0 kGy, respectively. Despite the fact that irradiation assisted in faster declines of listeriae during fermentation, these bacteria showed a strong tailing during ripening, which was more pronounced in sausages irradiated at 4 kGy. As a consequence, survival of *Listeria* in 28-day sausages irradiated at 2 or 4 kGy was ca. 2 log cfu g^{-1} and similar ($P > 0.05$) to that in non-irradiated samples. Irradiation showed promise for controlling *E. coli* O157:H7 and, to a lesser extent, *L. monocytogenes* in fermented sausages.

Keywords: Irradiation; *Listeria*; *Escherichia coli* O157:H7; Fermented sausage; Salami

1. Introduction

Listeria monocytogenes and *Escherichia coli* O157:H7 are important meat-borne pathogens (Lou & Yousef, 1999; Park, Worobo, & Durst, 1999) and may survive in traditional uncooked dry fermented sausages (Clavero & Beuchat, 1996; Glass, Loeffelholz, Ford, & Doyle, 1992; Samelis & Metaxopoulos, 1999). Especially *E. coli* O157:H7 has a high acid resistance (Conner & Kotrola, 1995) and has been the cause of recent hemorrhagic colitis outbreaks associated with consumption of fermented sausages in the US (CDC, 1995; Tilden et al., 1996). As a response, USDA-FSIS announced a mandate to require sausage manufacturers to guarantee processes that result in a 5-log reduction of *E. coli* O157:H7 in the final product (Billy, 1997). Several validation studies undertaken to adopt this mandate have shown that typical starter-mediated, fast-acid sausage fermentations deliver 1 to 3-log reductions only (Calicioglu, Faith, Buege, & Luchansky, 1997; Getty, Phebus, Marsden, Fung, & Kastner, 2000; Hinkens et al., 1996; Riordan et al., 1998; Tomicka, Chen, Barbut, & Griffiths, 1997). Thus, to comply with the 5D requirement, post-fermentation heat treatments are necessary (Getty et al., 2000). Other studies have shown survival of inoculated *L. monocytogenes* in various European and American-style fermented

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sausages (Buncic, Paunovic, & Radisic, 1991; Farber, Daley, Holley, & Usborne, 1993; Johnson, Doyle, Cassens, & Schoeni, 1988; Lahti, Johansson, Honkanen-Buzalski, Hill, & Nurmi, 2001; Nissen & Holck, 1998). In Greece, *L. monocytogenes* along with other *Listeria* spp. have been found to persist in almost any batter at formulation of dry salami (Samelis & Metaxopoulos, 1999; Samelis, Metaxopoulos, Vlassi, & Pappa, 1998). Although a 7-day fermentation was sufficient to eliminate listeriae (Samelis et al., 1998), their high presence in batters is a concern that requires preventive measures to be taken by Greek salami manufacturers.

Post-fermentation heat treatments are not a viable option for traditional European sausages, including Greek dry salami, because of undesirable effects of heating on product authenticity and sensorial quality. Thus, alternative methods are needed to achieve an equivalent level of safety; the use of properly decontaminated pathogen-free raw materials for dry sausage manufacture could be a feasible option. However, chemical decontamination technologies of animal carcasses currently applied commercially in the US (Huffman, 2002) are still not permitted in Europe. In addition, spraying of carcasses or primal meat cuts with organic acids or other chemicals may cause sensorial changes and increase pathogen stress adaptation, resistance and virulence on decontaminated meat (Samelis & Sofos, 2003). On this basis, irradiation and high hydrostatic pressure may be better options for pathogen control on raw meat trimmings or sausage batters compared to chemical (acid) decontamination (Prochaska, Ricke, & Keeton, 1998). Although irradiation has been studied for about 50 years and its bactericidal effects have been demonstrated in fresh and cooked meat products (Farkas, 1997, 2001; Lee, Sebranek, Olson, & Dickson, 1996; Sommers et al., 2004), scanty information exists on its use in fermented sausages (Dickson & Maxcy, 1985), especially following the emergence of *E. coli* O157:H7 as a major pathogen of concern in these products (Johnson, Sebranek, Olson, & Wigand, 2000). Therefore, this study aimed at evaluating ionizing radiation as a decontamination technology against *Listeria* spp. and *E. coli* O157:H7, which may potentially be present on raw meat and fat trimmings used for manufacture of dry fermented sausages.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Four strains of *Listeria innocua* (LMG 11387, L11, L12, and L14), one non-virulent strain of *L. monocytogenes* (No. 10, serotype 4ab, kindly provided by Prof. J. Farkas, Szent Istvan University, Budapest, Hungary), and *E. coli* O157:H7 strain ATCC 43888 (human feces isolate, not producing Shiga-like toxin I and II) were used. Strains L11, L12, and L14 were Greek sausage meat isolates from our collection, sharing an API *Listeria* (BioMerieux, Marcy-l'Etoile, France) profile with typical *L. monocytogenes*, except for their positive DIM reaction, a differentiating characteristic of *L. innocua*. All strains were selected to be non-pathogenic, but closely related to pathogenic *L. monocytogenes* and *E. coli* O157:H7, because fermented sausages of this study were processed under commercial conditions, and thus, the inclusion of pathogens in real in-plant experiments was ethically and scientifically prohibited. All strains were kept frozen (−30 °C) in trypticase soy broth (TSB) (BBL, Becton Dickinson, Sparks, MD) with 20% (w/v) glycerol. They were activated by transferring 0.05 ml of stock culture into 10 ml of TSB plus 0.6% yeast extract (TSBYE), incubating at 30 °C for 24 h. Strains were subcultured twice before use in the experiments.

2.2. Preparation and inoculation of sausage meat trimmings

Artificially contaminated sausage samples were processed in a local meat processing plant (VIKI S.A., Filippiada, Greece). First, trimmings of pork and beef meat and pork back fat were taken from respective frozen (−25 °C) stock batches of raw materials, pre-tempered at −10 °C, and mixed at a ratio of 40:30:30. Mixed trimmings (24 kg of total weight) were coarse cut (10 mm in diameter) in a 50-kg pilot cutter. The mixture was inoculated with approximately 6 log cfu g^{-1} of *Listeria* spp. and *E. coli* O157:H7, respectively. To prepare the inocula, 10-ml TSBYE cultures of each *Listeria* strain and one 50-ml TSBYE culture of *E. coli* O157:H7 strain ATCC 43888 were incubated at 30 °C for 24 h, mixed, and centrifuged at 3000 rpm for 15 min. Cells were harvested, washed with sterile Ringer solution and re-suspended in 100 ml of diluent. This composite culture was inoculated into the mixed trimmings, and the cutter was rotated for 30 s to evenly distribute the inoculum in the meat. Then, the inoculated trimmings were divided in three equal parts of 8 kg each, corresponding to the number of irradiation treatments applied. Each part was further divided in 2-kg portions, which were vacuum packaged, pressed to form flat blocks of ca. 6 cm in thickness, and stored in a freezer (−25 °C) for one week prior to irradiation. Freezing was done in order to simulate common industrial practices for fermented sausage manufacture in Greece (e.g., most manufacturers use frozen pork and beef meat and pork back fat trimmings thawed just before sausage formulation), as well as to facilitate irradiation.

2.3. Irradiation of meat trimmings

Frozen inoculated trimmings were transferred in insulated polystyrene boxes to the irradiation plant