Reduction of *Escherichia coli* O157:H7 during manufacture and ripening of Italian semi-dry salami

Elena Dalzini,† Elena Cosciante-Cunico,‡ Paola Monastero,† Chiara Sfameni,‡ Enrico Pavoni,‡ Paolo Daminelli,† Marina-Nadia Losio,‡ Andrea Serraino,‡ Giorgio Varisco†

†Centro di Referenza Nazionale per i RischiEmergenti in Sicurezza Alimentare, Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna B. Ubertini, Brescia; ‡Reparto di Microbiologia, Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna B. Ubertini, Brescia; †Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano dell’Emilia (BO), Italy

**Abstract**

In order to simulate a contamination at the processing plant, one batch of freshly-processed salami batter (20 kg) was inoculated (1% v:w) with 5 log colony forming unit (CFU)/g of a multi-strain cocktail of two strains of *Escherichia coli* O157:H7 (registered and wild strain). Another batch was inoculated (1% v:w) with sterile physiological saline solution and used to check the lactic acid bacteria (Lab) behaviour and the changes of physico-chemical parameters (pH and a_w). Both batches were then processed to obtain a semi-dry salami (Hungarian-style): microbiological and physico-chemical properties were monitored during 94 days of ripening. During the manufacturing process, the levels of pathogen decreased of about 2.18 log CFU/g with respect to the initial inoculated levels. The behaviour of the indigenous bacteria such as Lab and the physico-chemical properties can help to determine the fate of pathogens throughout processing.

**Introduction**

*Escherichia coli* strains isolated from human diseases have been grouped into at least six different diarrhoeagenic *E. coli* groups based on specific virulence factors and phenotypic traits. Strains of Verotoxigenic *E. coli* (VTEC) have become emergent foodborne human pathogens since the first documentation of VTEC O157:H7 as the infective agent in an outbreak in 1982 (Riley et al., 1983). The first recognised community outbreak of O157:H7 in Europe occurred in the United Kingdom in the summer of 1985 and further outbreaks and sporadic cases have been reported throughout Europe ever since (Gillespie et al., 2005). In Europe and all over the world several outbreaks of *E. coli* O157:H7 caused by fermented sausage were reported: in 1994 in the USA, 20 cases were reported and were associated with consumption of dry-cured sausage (Alexander et al., 1995); in Canada (1998-1999) 182 cases were reported and associated with salami and Genoa salami consumption (Williams et al., 2000; MacDonald et al., 2004); in Sweden (2002) a total of 39 cases was associated with fermented sausages consumption (Sartz et al., 2008). In Italy, a family outbreak of *E. coli* O157:H7 was microbiologically associated with consumption of dry-fermented salami made with pork meat and produced in a local plant (Comedera et al., 2007).

These epidemiological data evidence that foodborne disease from dry sausages cannot be underestimated and efforts should be made to control contamination at slaughter level and to limit bacterial growth at processing stage (Barbuti and Parolari, 2002). Based on significant contamination rates in raw meat, evaluating the behaviour of *E. coli* O157:H7 during processing of meat products appear to be another important step to achieve microbial safety (Barbuti and Parolari, 2002).

The objective of the study was therefore to evaluate the behaviour of *E. coli* O157:H7 during processing and ripening of salami, a typical Italian dry-cured sausage.

**Materials and Methods**

**Bacterial strains**

A reference strain (*E. coli* O157:H7 ATCC® 35150™) and a field isolate of *E. coli* O157:H7 (Ec 72209) isolated from dry sausage and stored in the culture collection of the Veterinary Epidemiology Centre of Brescia, were used in this experimental study. Each strain, previously kept frozen, was transferred (2% inoculum) into brain heart infusion (BHI) broth and incubated at 37°C for 24 h in aerobic conditions. The cultures were centrifuged for 60 min at 4°C at 4000 g (Jouan centrifuge CR422; Jouan Inc., Winchester, VA, USA); the pellet was washed with sterile physiological solution (H_2O with 0.9% NaCl), centrifuged as previously described and re-suspended in sterile physiological solution. For each strain, the culture concentration was checked by plate counting on BHI agar. The two strains were combined in equal volumes, serially diluted and inoculated in salami batter to reach approximately 5 log colony forming units (CFU)/gram.

**Manufacture of salami, inoculation of salami batter and sampling times**

A batter of 40 kg was prepared by mixing minced pork meat (50%), beef meat (25%) and pork lard (25%) refrigerated at 0-7±2°C. During mixing, potassium nitrate (E 252) and sodium nitrate (E 250) (0.03% both), sodium chloride (3%), dried skimmed milk (2.5%), saccarose, sodium ascrobate, black and white pepper and garlic were added.

The batter was divided into two batches of 20 kg each. The first batch was inoculated with the multi-strain pathogen cocktail (1% v:w) to obtain a final concentration of about 5 log CFU/g; the second batch was inoculated with sterile physiological saline (1% v:w) to obtain control samples.

After inoculation the batter was mixed at room temperature (22±2°C) for 5 min and stuffed into reconstructed casings (105 mm bore, about 2-3 kg weight for each piece) and matured according to the following procedure: 12 h at 23°C with no control of relative humid-
Table 1. Changes of Escherichia coli O157:H7, lactic acid bacteria, pH and aw throughout the ripening of semi-dry salami.

| Parameter | Batter | Salami at different ripening times (days) |
|-----------|--------|----------------------------------------|
|          | 6      | 14 | 20 | 42 | 60 | 75 | 82 | 94 |
| Ec<sup>+</sup> (log CFU/g) | 5.17±0.06<sup>A</sup> | 4.75±0.05<sup>B</sup> | 4.66±0.10<sup>BC</sup> | 4.52±0.05<sup>BC</sup> | 4.38±0.09<sup>BC</sup> | 3.20±0.06<sup>D</sup> | 3.06±0.13<sup>D</sup> | 2.63±0.08<sup>E</sup> | 2.99±0.26<sup>D</sup> |
| Lab<sup>+</sup> (log CFU/g) | 6.21±0.13<sup>A</sup> | 8.58±0.19<sup>B</sup> | 8.47±0.14<sup>B</sup> | 8.51±0.11<sup>B</sup> | 8.39±0.09<sup>B</sup> | 8.17±0.06<sup>B</sup> | 8.12±0.05<sup>B</sup> | 7.7±0.16<sup>CD</sup> | 7.46±0.41<sup>D</sup> |
| pH | 5.65±0.02<sup>A</sup> | 4.95±0.03<sup>B</sup> | 4.92±0.05<sup>B</sup> | 4.87±0.03<sup>B</sup> | 5.05±0.05<sup>BC</sup> | 5.57±0.20<sup>B</sup> | 5.59±0.07<sup>B</sup> | 5.58±0.17<sup>B</sup> | 5.44±0.06<sup>B</sup> |
| aw<sup>+</sup> | 0.956±0.007<sup>BC</sup> | 0.925±0.021<sup>BC</sup> | 0.940±0.009<sup>BC</sup> | 0.921±0.010<sup>BC</sup> | 0.938±0.002<sup>BC</sup> | 0.916±0.013<sup>BC</sup> | 0.901±0.002<sup>BC</sup> | 0.906±0.021<sup>BC</sup> | nd |

Ec<sup>+</sup>, Escherichia coli O157:H7; Lab, lactic acid bacteria; aw<sup>+</sup>, water activity; nd, not determined. Data represent the average values ± standard deviation of three replicates samples. *Evaluated in contaminated samples; †Evaluated in control samples. *<sup>A</sup>Means with different uppercase letters within a row for each parameter are significantly different (P<0.05).
would increase the safety of the sausages. Since a large number of different sausage types exist, differing profoundly not only in pH, salt content, water content and recipes, but also in production conditions like fermentation temperature and maturation time, such differences must be taken into consideration when trying to validate the safety of specific fermented sausage productions. To ensure that the fermentation and drying process are efficient to reduce or eliminate pathogens, procedures should be validated to demonstrate that they achieve established reduction for specific organisms. Data reported in the present work will be useful for food manufacturers that produce ready-to-eat meat products with similar characteristics. Still, the control of hygienic quality of meat used for salami production should be of primary importance to any producer.

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