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

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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 (Conedera *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.

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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 ascorbate, 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-

ity (RH), 24 h at 20°C at an average RH of about 70-85% followed by a gradual decrease of temperature to 11-12°C and increase of RH to 85% in 10 days. Salami were then left to ripen at 11-12°C and 75-85% RH up to 94 days from manufacture.

Microbiological and physico-chemical analysis

Samples were collected from contaminated and control batches in three replicates for each sampling by the batter (day 0) and by salami at 6, 14, 20, 42, 60, 75, 82 and 94 days during ripening. For enumeration of *E. coli* O157:H7 in the contaminated batch, 25 g of batter/salami were transferred into plastic one-chamber filter stomacher bags (NEOMED, London, UK) and homogenised 1:3 w:v in sterile peptone water (PW) (CONDA, Madrid, Spain) for 3 min in a Stomacher 400 blender (Seward Medical, London, UK). Decimal dilutions in sterile PW were prepared from each bag and the appropriate dilutions were surface-plated (100 L) onto duplicate plate of selective-differential Mac Conkey Sorbitol Agar added with Cefixime Tellurite supplement (CT-SMAC) (Diagnostic Systems, Holzheim, Germany). Typical pathogen's colonies were counted after aerobic incubation of plates at 37°C for 24 h. To verify the absence of *E. coli* O157:H7 in the raw materials, the presence of *E. coli* O157:H7 was also investigated in the control batch.

Mesophilic lactic acid bacteria (Lab) were enumerated on control samples by pouring plates of 1 mL of appropriate dilution in de Man, Rogosa and Sharpe agar (MRS) (Microbiol Diagnostici, Cagliari, Italy) and incubating at 30°C for 72 h. Physico-chemical analyses were performed on 10 g of control samples; the pH was determined using a HI 223 Calibration checkTM Microprocessor pH meter (Hanna Instruments, Woonsocket, RI, USA) and the water activity (a_w) was measured at 25°C with the a_w recorder AquaLab, series 3, Model TE (Decagon Devices, Inc., Pullman, WA, USA) in accordance with ISO 21807:2004 (ISO, 2004).

Statistical analysis

Microbiological count results were expressed as CFU/g and reported as log CFU/g. The average and standard deviations of microbial counts and physico-chemical values were determined from the average of three samples at each sampling time for each batch. Analysis of variance (ANOVA) was carried out to evaluate the difference of pH, a_w and microbial counts at different storage time. Significance was statistically analysed by Student *t*-test at a 95% confidence interval ($P<0.05$) using R statistical software version 2.7.0 (R Development Core Team, 2008).

Results

Results of *E. coli* O157:H7 and Lab count, and physico-chemical properties of the batter and salami during the ripening are shown in Table 1.

The microbiological and physico-chemical properties of salami batter were found to be significantly different from those measured in salami. A significant ($P<0.05$) increase of Lab count was observed during the first 6 days of ripening (6.21 to 8.58 Log CFU/g); no significant differences were observed up to the 75th day of ripening and a moderate but significant decrease ($P<0.05$) was observed at 82 and 94 days of ripening.

A decrease of the pH value was observed in the first six days of ripening from an initial value of 5.65±0.02 to 4.95±0.03 followed by an increase after 42 days of ripening (Table 1). The a_w values showed a moderate decrease during seasoning from an initial value of 0.956±0.007 to 0.906±0.021 at the end of ripening (Table 1).

Examination of not contaminated samples at the beginning of shelf life revealed the absence of natural *E. coli* O157:H7 contamination in the batter mix. The average values of *E. coli* O157:H7 log counts in contaminated batches of salami are shown in Table 1. Starting from values of 5.17±0.06 log CFU/g in the batter, *E. coli* count decreased to 4.75±0.05 log CFU/g in the first 6 days of ripening. A gradual decrease of *E. coli* O157:H7 count was observed throughout the ripening stage of the salami, until reaching a level of 2.99±0.26 log CFU/g at the end of the ripening.

Discussion

An overall 2.18 log/CFU reduction was observed in *E. coli* O157:H7 count during production and ripening of salami. Holck and colleagues (2011) recently reviewed several studies carried out to determine the reduction of VTEC during the production of fermented sausages, in which ingredients or production parameters varied systematically (Riordan *et al*., 1998; Casey and Condon, 2000; Heir *et al*., 2010; Duffy and Vanderlinde, 2000). In these studies, different sausages, dry and semi-dry, have been investigated, looking at the effects of varying pH, nitrite concentration, fermentation temperature, degree of drying and ripening time. The main factors influencing the reduction of VTEC in fermented sausages appeared to be the temperature of fermentation and rapid drop of pH; salt content, water content, nitrite and recipes may also have an influence (Holck *et al*., 2011). Previous works reported an *E. coli* O157:H7 reduction during production of salami, pepperoni and various other fermented sausages of about 1-2 log, although deeper reductions are also observed (Nissen and Holck, 1998; Faith *et al*., 1997; Montet *et al*., 2009). The pH of sausages included in the studies mentioned above, varied from <4.8 to 5.2 and fermentation temperatures were in the range of 18 to 36°C, which is comparable to what reported in this study.

Conclusions

As a general remark, no single parameter appeared to influence survival of *E. coli* to such an extent that it could be adjusted to give completely safe sausages. Fermentation temperature appeared to be an important factor. An optimal combination of different hurdles

Table 1. Changes of *Escherichia coli* O157:H7, lactic acid bacteria, pH and a_w throughout the ripening of semi-dry salami.

Parameter	Batter	Salami at different ripening times (days)							
		6	14	20	42	60	75	82	94
Ec° (log CFU/g)	5.17±0.06^{A}	4.75±0.05^{B}	4.66±0.10^{BC}	4.52±0.05^{BC}	4.38±0.09^{C}	3.20±0.06^{D}	3.06±013^{D}	2.63±0.08^{E}	2.99±0.26^{D}
Lab° (log CFU/g)	6.21±0.13^{A}	8.58±0.19^{B}	8.47±0.14^{B}	8.51±0.11^{B}	8.39±0.08^{B}	8.17±0.06^{BC}	8.12±0.05^{BC}	7.7±0.16^{CD}	7.46±0.41^{D}
pH$^{\#}$	5.65±0.02^{A}	4.95±0.03^{B}	4.92±0.05^{B}	4.87±0.03^{B}	5.05±0.09^{C}	5.57±0.20^{C}	5.59±0.07^{C}	5.58±0.17^{C}	5.44±0.06^{C}
a_w $^{\#}$	0.956±0.007^{A}	0.925±0.021^{AB}	0.940±0.009^{AB}	0.921±0.001^{AB}	0.938±0.002^{AB}	0.916±0.013^{BC}	0.901±0.002^{C}	0.906±0.021^{BC}	nd

Ec, *Escherichia coli* O157:H7; Lab, lactic acid bacteria; a_w, water activity; nd, not determined. Data represent the average values±standard deviation of three replicates samples. °Evaluated in contaminated samples; #evaluated in control samples. $^{A-E}$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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