International Journal of Food Microbiology 262 (2017) 99–106

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Assessment of *Salmonella* survival in dry-cured Italian salami

S. Bonardi[a,*], I. Bruini[a], L. Bolzoni[b], P. Cozzolino[c], M. Pierantoni[c], F. Brindani[a], P. Bellotti[b], M. Renzi[d], S. Pongolini[b]

[a] Department of Veterinary Science, Unit of Food Inspection, University of Parma, Via del Taglio 10, 43126 Parma, Italy
[b] Risk Analysis Unit, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Sezione di Parma, Strada dei Mercati 13/A, 43126 Parma, Italy
[c] National Veterinary Service, Local Unit of Parma, Via Vasari 13/A, 43126 Parma, Italy
[d] Department of Veterinary Science, Unit of Livestock Science, University of Parma, Via del Taglio 10, 43126 Parma, Italy

ARTICLE INFO

Keywords:
Salmonella
Pork
Dry-curing
Real-time PCR
MPN enumeration

ABSTRACT

The inactivation of *Salmonella* during curing of Italian traditional pork salami was investigated. A total of 150 batches of ground raw meat (GRM) used for salami manufacturing by four producers were tested for *Salmonella* by real-time PCR followed by ISO 6579 cultural confirmation and MPN enumeration. Salami produced with *Salmonella* positive GRMs were re-tested at the end of their curing period. Aw, pH and NaCl content were also measured. Detection of *Salmonella* was performed testing both 25 and 50 g of the samples.

By Real-Time PCR 37% of the GRMs resulted positive, but cultural detection of *Salmonella* was obtained in 14% of the samples only. *Salmonella* enumeration ranged from 31 MPN/g to < 1.3 MPN/g. The difference between testing 50 g and 25 g of the samples was statistically significant (p value ≤ 0.01). In particular, ISO-50 g detected *Salmonella* in 100% of all positive samples, *vs.* 62% of ISO-25 g. Salami made of the contaminated GRMs were 29% *Salmonella*-positive, as most batches of salami produced with *Salmonella*-positive GRMs resulted negative after regular curing (20–48 days). Overall, 13% of salami produced with *Salmonella*-contaminated GRMs were positive. They belonged to six batches, which turned out negative after prolonged curing ranging between 49 and 86 days. *Salmonella* enumeration in salami ranged from 8.7 MPN/g to < 1.3 MPN/g. Unlike GRMs, no significant difference was observed between the ISO-50 g and the ISO-25 g in detecting *Salmonella* in cured salami (p value: > 0.05).

The most common *Salmonella* serovars in GRMs were Derby (52%), Typhimurium monophasic variant 4, (Barbuti et al., 1993), 12:i:- (19%) and Stanley (10%). *Salmonella* Derby (56%), London, Branderup, Panama (13%, respectively) and Goldcoast (6%) were most frequent in cured salami. The study showed negative correlation between real-time CT values and cultural confirmation of *Salmonella*, as well as the importance of sample size for *Salmonella* detection. Among considered factors with possible effect on the occurrence of *Salmonella* in salami, statistical analysis revealed a role for aw in salami and for *Salmonella* load in GRMs, while pH and NaCl content did not significantly affect the probability of finding *Salmonella* in dry-cured salami in the context of this study. In particular the lower aw values due to longer curing were associated with lower *Salmonella* presence in traditional dry-cured salami.

1. Introduction

Italian salami are dry fermented sausages which have been produced for centuries with a variety of ingredients and manufacturing processes. Dry fermented sausages from Mediterranean countries are usually air dried, due to the favourable climate, and rarely smoked. Pork meat is the main ingredient and fungal starter cultures may be used on the external surface imparting a complexity of flavours to the product (Talon et al., 2004).

The use of sodium chloride (NaCl) is essential in dry fermented sausages to solubilize proteins and emulsify fat. Furthermore, NaCl can control the growth of undesirable bacteria responsible for spoilage of meat and pathogenic bacteria (King et al., 2016). The usual amount of added salt is generally between 2 and 4% by weight (Ockerman and Basu, 2007) but its concentration increases in final products due to the drying process (Zanardi et al., 2010). In addition to NaCl, other salts are generally added to the pork and fat mixture, namely nitrates (maximum 150 mg/kg) and nitrites (maximum 150 mg/kg) to inhibit *Clostridium botulinum* (Hospital et al., 2016), enterobacteriaceae and enterococci (Coloretti et al., 2008) and to favour the red colour of cured meat

* Corresponding author at: Department of Veterinary Science, University of Parma, Via del Taglio, 10, 43126 Parma, Italy.
E-mail address: silvia.bonardi@unipr.it (S. Bonardi).

http://dx.doi.org/10.1016/j.ijfoodmicro.2017.09.016
Received 8 February 2017; Received in revised form 10 August 2017; Accepted 24 September 2017
Available online 28 September 2017
0168-1605/

(Villaverde et al., 2014).

Salami manufactured in the Emilia-Romagna region of Northern Italy, where this study was conducted, are generally made with pork only, and they have coarsely ground meat and 3–4 mm size cubes of fat. Fresh meat is obtained from shoulder and belly and fat is normally pork backfat. After grounding, salt, whole peppercorns and garlic are added in traditional products. Some formulations include sugars and starter bacterial cultures. Salami are then stuffed into a pork casing and commonly aged for 20 to 40 days according to their size (Mataragas et al., 2015a, 2015b).

Salmonella ranks second among pathogens reported in the European Union (EU) as causative agents of human zoonotic diseases (EFSA and ECDC, 2016). In 2014, pork meat and products thereof were responsible for 9.3% of 225 foodborne outbreaks caused by *Salmonella,* thus representing the type of meat most frequently associated with salmonellosis in humans (EFSA and ECDC, 2015). In Italy, consumption of salami was recently associated with both clustered and sporadic cases of salmonellosis due to S. Goaldcost (Scavia et al., 2013), *S.* Manhattan (Scaltriti et al., 2015), *S.* Typhimurium and *S.* Typhimurium monophasic variant (Andreoli et al., 2017; Luzzi et al., 2007). Other EU countries confirmed *Salmonella* outbreaks linked to salami, like Sweden in 2005, Norway in 2006 and Denmark in 2010 (Emberland et al., 2006; Hjertqvist et al., 2006; Kuhn et al., 2011).

Salmonella survival in cured meat products depends on dry-curing and physicochemical conditions created by several parameters, which interact all together, like salt, nitrite, pH, water activity and temperature. For example, water activity decrease is a key factor for *Salmonella* inactivation, but its effect depends also on contemporary pH decrease, as well as salt and nitrite concentration (Messier et al., 1989). Different studies were performed on Italian traditional salami, which addressed the fate of *Salmonella* under experimental conditions through artificial contamination (Mataragas et al., 2015a, 2015b; Nightingale et al., 2006). In these studies, composition and physicochemical conditions of salami were specified. On the contrary, our study aimed to assess the behavior of *Salmonella* in traditional salami, characterized by low standardization of production parameters, in field conditions. In this context, to cover the variability of the production process, a large number of batches distributed among different producers were included in the study with the purpose of assessing the effects of the basic physicochemical parameters (pH, $a_{w,}$ NaCl) regardless of the heterogeneity of the actual products on the market.

In the EU, microbiological food-safety criteria are set by the Regulation EC 2073/2005 (European Commission, 2005), which identifies culture-based ISO methods as the analytical reference methods. ISO 6579 is the standard for *Salmonella* detection in foods. This method relies on several cultural steps and requires > 5 days for conclusive results in case of positive samples. This is why, to meet the needs of the food industry, the same Regulation allows the use of alternative methods, generally more convenient and faster, under specified conditions. PCR-based methods are among available alternatives and several studies have been performed to assess real-time PCR protocols for the rapid and sensitive detection of *Salmonella* in foods in < 24 h (Delibato et al., 2014; Rodriguez-Lazaro et al., 2014).

Based on the above considerations, our study focuses on characteristic salami from Emilia Romagna and the aims were: *i*) to determine the prevalence of *Salmonella* in the mixtures of minced raw pork and fat used for salami production, *ii*) to assess the effect of curing on the fate of *Salmonella* in the end product by testing the batches of salami manufactured with the *Salmonella*-positive raw mixtures, *iii*) to investigate the influence of physicochemical parameters on *Salmonella* contamination in dry-cured salami, *iv)* to measure the load of *Salmonella* in dry-cured salami, *v*) to assess the effect of sample size on the probability of *Salmonella* detection, and *vi*) to investigate the proportion of real-time PCR positive samples confirmed by ISO 6579.

2. Material and methods

2.1. Sample collection

From April to December 2015, 150 samples of ground raw mixtures (GRM) made of ground pork and fat, collected from 150 different batches of starting material for salami processing, were tested for *Salmonella.* Only pig meat and pig backfat were used for the salami manufacturing. The samples were collected in four production plants, here identified as A (47 samples), B (23 samples), C (11 samples), D (69 samples) located in Emilia-Romagna region, Northern Italy. The plants were included in the study based on their willingness to take part to the study (five were asked to participate) and the different number of samples collected from each plant was proportional to its production capacity. Meat and fat suppliers of the four plants were many and, often, meat and fat from more than one supplier were mixed in the same batch of GRM. In our study the GRM samples of the four companies were collected before addition of other ingredients and additives (salt, nitrites, nitrates, ascorbates, black pepper) to avoid potential interference with *Salmonella* detection. Nitrates and nitrates were added in compliance with the limits set by Regulation EU No 1129/2011 on food additives. Curing was performed at temperatures below 20 °C, specifically in the range of 12–18 °C.

Whenever a GRM was positive for *Salmonella*, the derived batch of salami was tested for the pathogen at the end of its curing period which ranged from 20 to 48 days according to the different producers' protocols. Five salami (5 sampling units) per batch were tested. Since 21 GRM samples were positive for *Salmonella*, 21 batches of salami (for a total of 105 salami) were analyzed at the end of their curing. The total number of tested salami was 140, because 6 batches of salami out of 21 resulted contaminated by *Salmonella*, and were re-tested after a prolonged curing period of 21–38 days (for an additional 5 salami per batch). Furthermore, since one batch was still positive, 5 more salami were analyzed after an additional curing of 8 more days (total curing duration: 62 days) (Table 1). The values of pH and a_w and the content of NaCl were determined in the 140 tested salami.

2.2. Salmonella detection in ground raw mixture (GRM) and salami

Detection of *Salmonella* in both GRM and salami was performed by real-time PCR followed by microbiological confirmation. A pre-enrichment broth was prepared suspending 25 g of sample in 225 ml of Buffered Peptone Water (BPW, Oxoid, Basingstoke, UK) and homogenizing for 2 min in a Stomacher blender. After 18 ± 2 h at 37 ± 1 °C DNA was extracted from 1 ml of the pre-enrichment culture using SureFood PREP *Salmonella* Kit (R-Biopharm, Darmstadt, Germany) and PCR master-mix was prepared with SureFast *Salmonella* ONE Kit (R-Biopharm) for a final volume of 25 µl containing 5 µl of template DNA. PCR reactions were run on a Mx3005P QPCR System (Agilent Technologies, Italy) with the following thermal program: a cycle of DNA polymerase activation of 5 min at 95 °C followed by 45 amplification cycles of 15 s at 95 °C and 30 s at 60 °C (annealing-extension step). The samples with a cycle threshold (CT) value lower than 40 were considered positive. The other samples were considered negative for *Salmonella*.

PCR positive samples underwent microbiological testing by ISO 6579:2002 starting from aliquots of 25 g and 50 g, the latter being re-sampled from the meat matrix, the former coinciding with the pre-enrichment step used for PCR. All meat samples were stored at 3 °C (± 1 °C) for up to 24 h before resampling. Presumptive isolates of *Salmonella* were assayed with O-omnivalent *Salmonella* serum by slide agglutination (Denka Seiken, Tokyo, Japan). Biochemical identification to the genus level was carried out with API® 20E system (bioMérieux, Marcy l'Etoile, France).