Non-thermal inactivation of *Listeria* spp. in a typical dry-fermented sausage: "Bergamasco" salami

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Abstract

Aim of the present study was the evaluation of the growth potential of *Listeria* spp. inoculated in the typical North Italian dry fermented sausage "Bergamasco" salami during its production. As it was necessary to carry out the challenge test in the production line of the industry, according to the guidelines of the European Reference Laboratory for *Listeria monocytogenes*, a non-pathogenic "surrogate" microorganism was used: for the inoculum, two strains of *Listeria innocua* (1 ATCC, 1 strain isolated from a similar substrate) were used. The inoculation of the samples occurred during grinding and mixing of the sausage mass, before the filling. To avoid cross-contamination, the control samples were produced before the contaminated ones. After the dripping, salamis were subjected to the normal production process (drying and maturation in five steps at specific temperatures and humidity rates). The inoculated products were subjected to the enumeration of *Listeria* spp. at T0 (day of inoculation) and at T4 (post-drying), and every 10 days during curing (T10, T20, T30, T40, T50, T60, T70, T80 and T90), as this salami is generally sold as whole piece with varying levels of curing (from T20 to T90). Since the product may be cut in half and vacuum-packed, at each of the times starting from T20, half salami was vacuum-packed and stored for 30 days at 12°C, at the end of the which *Listeria* spp. enumeration was performed again. At all times and for each type of samples of each of the three batches, the enumeration of the natural microflora (Total Viable Count, lactic acid bacteria, *Pseudomonas* spp., *Enterobacteriaceae*) and the determination of water activity and pH were performed on control samples. The product was characterized by a high concentration of microflora (8-8.5 Log UFC/g), consisting mainly of lactic acid bacteria, added to the mixture at the beginning of the production process. The pH showed a decrease over time, expected for this type of products, due to the development of lactic acid bacteria (final pH: 5.42-5.55). The water activity reached values able to inhibit the development of *Listeria* spp. (final a_w: 0.826-0.863). *Listeria* counts in the tested batches of "Bergamasco" salami showed the absence of significant growth in the product with a reduction of loads if compared to T0, between -0.59 and -1.04 Log CFU/g. Even in the samples subjected to vacuum packaging and storage at 12°C, the absence of significant increase of lactic acid bacteria in the product was highlighted with further decrease of bacterial loads (-0.70/-0.79 Log CFU/g if compared to T20). Considering the worst case scenario (thus the batch with the highest growth potential), in the products stored in the curing room at 14-16°C, at humidity of 80% and in the samples stored at 12°C and vacuum packaged, the threshold indicated by the EURL Lm guidelines (+0.5 Log CFU/g) for the growth of *Listeria* spp. was not reached, allowing to classify "Bergamasco" salami in the category 1.3 of the EC Reg. 2073/2005 as "Ready-to-eat food unable to support the growth of *Listeria monocytogenes*".

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Key words: dry-fermented sausage; growth potential; *Listeria*; EURL Lm guidelines.

Contributions: the authors contributed equally.

Conflict of interests: the authors declare no conflict of interests.

Funding: none.

Received for publication: 14 February 2019.
Accepted for publication: 7 May 2019.

Italian Journal of Food Safety 2019; 8:8112
doi:10.4081/ijfs.2019.8112

Introduction

The presence of *Listeria monocytogenes (L. monocytogenes)* in dry fermented sausages is currently one of the main concerns for meat industry. Dry fermented sausages are Ready-to-Eat (RTE) products where the presence of this pathogen represents a potential risk for consumers, especially for susceptible populations. In fact, invasive listeriosis mainly occurs in immunocompromised patients, elderly people, pregnant women, young, unborn or newborns. In particular, pregnant women experience mild symptoms followed by abortion, stillbirth, premature birth or newborns subjected to bacteraemia and meningitis (Jackson *et al*., 2010; Silk *et al*., 2012).

Although relatively rare, invasive listeriosis is characterized by high fatality (16.7% in 2016) and hospitalization rates (97.7% in 2016) and it is considered of major concern (EFSA-ECDC, 2017). Several studies reported the presence of *L. monocytogenes* in Italian sausages with prevalence from 9 to 45% (Cantoni *et al*., 1988; Barbuti *et al*., 1989; Cantoni, 1991; De Cesare *et al*., 2007; Meloni, 2015) and concentration generally below 1 Log CFU/g, but in some sporadic cases higher than 3 Log CFU/g (Thevenot *et al*., 2005; De Cesare *et al*., 2007; Martin *et al*., 2011; Meloni, 2015). Outbreaks and sporadic cases of listeriosis are mainly associated with RTE foods, that are expected to be eaten without any application of killing process before consumption (Farber and Peterkin, 1991; FAO-WHO, 2001; EFSA-ECDC, 2017). According to EFSA-ECDC (2017), *L. monocytogenes* is more prevalent in 'RTE fish' with 10.3% of positive samples (1.7% above 100 CFU/g) and in 'RTE meat' with 2.07% of positive samples (0.43% above 100 CFU/g). Moreover, during the time period 2008-2015, non-compliance at processing ranged from 0.9% to 6.8% for RTE products of meat origin other than fermented sausages, and from 0% to 0.6% for RTE products of meat origin and fermented sausages. Contamination of dry-fermented sausages is mainly due to the presence of *L. monocytogenes* in raw materials (*e.g*. pork) (Thevenot *et al*., 2005), but its survival in the final product is frequent (Tompkin, 2002). The presence of the pathogen in the final products due to a contamination during slicing and packaging has been reported by Martin *et al*. (2011); theses authors detected relatively high prevalence rates of *L. monocytogenes* in the equipment (11.8%), raw materials (28.9%), and final products (15.8%), in small-scale Spanish factories producing traditional fermented sausages.

During fermentation and drying of sausages, *L. monocytogenes* is reported to decrease due to the combined action of several hurdles such as the reduction of water activity (a_w), and the competing effect of lactic acid microflora added to the mass (Bunčić *et al*., 1991). Mataragas *et al*. (2015) reported a linear decrease of *L. monocytogenes* in artificially contaminated Italian salami ("Cacciatore" and "Felino"), highlighting how pH and a_w resulted to be crucial parameters during fermentation. Also, Drosinos *et al*. (2006) reported a concentration reduction of 3-4 Log CFU/g after 28 days of drying in a Serbian fermented sausage.

The compliance with the limit of 100 CFU/g throughout the shelf-life of RTE products, is in charge to food business operators (Reg. EU 2073/2005). According to the European Reference Laboratory for *Listeria monocytogenes* (EURL Lm), the growth potential (d), as the difference between the *L. monocytogenes* concentration found at the end and the at the beginning of the shelf-life in Log CFU/g is one of the options to classify the product as able or unable to support the growth of *L. monocytogenes* (ANSES, 2014).

In particular, when d is > 0.5 Log CFU/g, food is classified as "Ready-to-eat food able to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes" (category 1.2), while if δ is ≤ 0.5 Log CFU/g, food is classified as "Ready-to-eat food unable to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes" (category 1.3).

Aim of the present study was to evaluate the growth potential of *L. monocytogenes* in "Bergamasco" salami in accordance with EURL Lm guidelines to satisfy the need of the food safety criteria for *L. monocytogenes* as reported in EU legislation.

Materials and Methods

Ingredients

The trial was conducted on "Bergamasco" salami produced starting from swine meat and fat, NaCl (2-2.5% was added to the mass), spices, sodium nitrite, potassium nitrate. A mixture of starter cultures was added to the salami mass before the filling, and was composed by *Staphylococcus carnosus*, *Pediococcus acidilactici* and *Lactobacillus plantarum*. The manufacturing company produced different sizes of the product, made with the same salami mass, thus the largest size (1 kg) was chosen as worst-case scenario, as it is characterized by a slower drying if compared to a salami of a smaller size.

Production process

The production process includes the following phases: reception of the cut meat and fat, storage in refrigerated rooms at 1-2°C, grinding and mixing of the ingredients, addition of starter cultures and other ingredients, filling, dripping for 2-5 hours at a temperature of 28±2°C (free relative humidity), drying and curing divided in 5 phases as follows: first phase for 12±2 hours at a temperature of 16±2°C and relative humidity of 60-65%; second phase for 24 hours at a temperature of 18±2°C and relative humidity of 65-70%; third phase for 24 hours at a temperature of 20±2°C and relative humidity of 75%, fourth phase for 24 hours at a temperature of 18±2°C and relative humidity of 75% and fifth phase that lasts up to the sale of the product, at a temperature of 14-16°C and relative humidity of 78-80%.

The product is sold as whole piece, starting from T20 (calculated from the day of production) to T80 days of aging. During sale, the product could also be cut in two halves and vacuum-packed, with an additional shelf life of 30 days.

Experimental protocol

The protocol was developed following the indications of the *EURL Lm technical guidance document for conducting shelf-life studies on* Listeria monocytogenes *in ready-to-eat foods* (ANSES, 2014).

Number of batches

The tested product has a certain variability especially regarding the raw ingredients (fresh pork); the availability of historical data on the characteristics of the product was limited, thus the challenge test was carried out on three batches of product.

Choice of Listeria *strains for the test*

As indicated in the EURL Lm guidelines, as it was necessary to conduct a challenge test directly at the production plant, a non-pathogenic "surrogate" microorganism, replacing *L. monocytogenes*, was used. The more similar candidate, from a metabolic and ecological point of view, was *Listeria innocua*: two strains were selected: *L. innocua* ATCC 33090 and a *L. innocua* strain previously isolated from a salami substrate.

Evaluation of growth potential of L. innocua *in broth*

In order to determine whether the selected strains had a growth capacity comparable to *L. monocytogenes*, the two strains of *L. innocua* were compared to two strains of *L. monocytogenes* belonging to the panel of strains provided by the National Laboratory of Reference for *L. monocytogenes* of the Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise (NRL Lm). These strains, identified as 12MOB045LM and 12MOB085LM, were the most suitable for the conduction of challenge tests on meat products, for their excellent growth rates under conditions of low temperature, acidic pH or low water activity (ANSES, 2013).

The four strains (2 *L. innocua* and 2 *L. monocytogenes*), kept frozen at -80°C in Microbank Cryogenic vials (Pro-Lab Diagnostics U.K., Merseyside, UK), were grown in TSB (Tryptone Soy Broth, Biogenetics, Ponte San Nicolò, Italy) at 37°C in order to reach the same growth phase; then they were subjected to enumeration by microscope and diluted in order to obtain the same starting concentration (~2 Log CFU/g). Each strain was then inoculated in duplicate in the following broths: TSB, TSB at pH 5.5 (adjusted with HCl), frequent pH value found in salami, and TSB with 3% NaCl in order to mimic the NaCl concentration of the salami mass. The broths were incubated at the following temperatures: 37°C (only the TSB broth, to verify the growth under optimal conditions), 20°C and 12°C. For each sample, optical density (OD) at 540 nm (6320D spectrophotometer, Jenway, Staffordshire, UK) was determined at T0 (immediately after inoculation) and after 24 h and 48 h of incubation.

Preparation of the inoculum and inoculation procedure

L. innocua strains were subcultured in TSB broth at 37°C for 18 h, in order to obtain strains at the early stationary growth phase. Then each strain was subcultured in a suspension obtained by homogenizing TSB broth with salami mass (in a 1:1 ratio), in order to pre-adapt the microorganisms to the substrate conditions. The suspensions were then incubated at 10-12°C for 3 days. Each of the bacterial suspensions was then enumerated (microscopic method) and diluted with sterile physiologic saline solution (NaCl 0.85%) in order to obtain the same concentration (4 Log CFU/ml); then, the two suspensions were mixed in equal quantity. The mixed suspension used for inoculation was subjected to microbial counts on Palcam agar (Biogenetics).

The inoculation of the samples was done during mixing of the salami mass, before the filling. The inoculum volume was 100 ml. This volume was adapted to the weight of the salami mass (10 kg for each batch), in order to avoid a change in the substrate conditions (1% of the total weight of the sample). The control samples were added with the same amount of sterile physiologic saline solution, in order to mimic the

bacterial inoculum. In order to avoid cross-contamination, the control samples were produced and filled before those artificially contaminated. The contaminated and control salami were kept at the production site for the whole duration of the trial, in order to apply the usual drying and ageing conditions. Samples were analyzed at T0 (salami mass was sampled after inoculation and mixing), at T4 (after drying), and every 10 days when the product was ready for sale, starting from the 20th day from the production (T20) and until the 90th day (T90); the product is generally sold with a curing period of no more than 70-80 days. As there is the possibility that the salami, during the retail phase, could be cut in two halves and vacuum-packed, for the samples of each batch from T20, half salami was vacuum-packed and stored for a period of 30 days. These sampling units were then stored at 12±1°C (thermal abuse condition intended to mimic retail and domestic storage).

Determination of the absence of Listeria *spp. in control samples*

To verify the absence of *Listeria* spp. in control samples, 3 non-inoculated sampling units of each batch were sampled in deep immediately after filling and subjected to detection of *Listeria* spp. In particular, 25 g of sample were diluted with 225 ml of Half Fraser broth, incubated at 37°C for 24-48 hours; afterwards a loop of the broth was streaked onto Palcam agar, and incubated at 37°C for 48 hours.

Challenge tests analyses

Inoculated samples were subjected to the enumeration of *Listeria* spp. in triplicate by diluting 1:5 the sample (10-15 g) in peptone saline solution (8.5 g/L NaCl, 1 g/L of peptone) and spreading on Palcam agar, incubated at 37°C for 48 h. Sampling times were T0, T4, T20, T30, T40, T50, T60, T70, T80 and T90. For vacuum packed salami, sampling times were T20+30, T30+30, T40+30, T50+30, T60+30, T70+30, T80+30 and T90+30.

Control samples were tested at the same time for the enumeration of Total bacterial mesophilic aerobic count (TVC-ISO 4833-2: 2013), *Pseudomonas* spp. (ISO 13720: 2010), *Enterobacteriaceae* (ISO 21528-2:2004), lactic acid bacteria (ISO 15214:1998), pH (by homogenization and dilution 1:5 with distilled water) and aw determination.

Calculation of growth potential

Results obtained from the enumeration of *Listeria* spp. have been transformed into Log CFU/g, and used to calculate the trend of the concentration in contaminated samples. According to the guidelines of the EURL Lm, the growth potential (δ) of *L. monocytogenes* in a food product is the difference between the logarithmic medians of the counts detected, respectively, at the end and at the beginning of the challenge test. Food is considered able to support *L. monocytogenes* growth when the δ value is greater than 0.5. The growth potential was calculated for each lot, using median values. Once the values were calculated for each of the 3 batches analysed, the highest δ value was chosen.

Results

Comparison between the growth of *L. innocua* strains and *L. monocytogenes* reference strains

L. innocua strains used for the test showed growth rates similar to those reported for *L. monocytogenes* reference strains; the mean optical density values measured in inoculated broths are shown in Table 1. The data measured at 12°C did not show any increase in optical density in 48h, and were not therefore reported in the table.

Determination of the absence of *Listeria* spp. and enumeration of microflora in control samples

The presence of *Listeria* spp. was never detected in control samples from the three batches analysed at T0. The results of the microbiological and chemical-physical analyses of batches 1, 2 and 3 are reported in Tables 2, 3 and 4, respectively.

As reported in the tables, the TVC of salami ranged from 5.55 to 8.97 Log CFU/g, and it was typical for this product, being substantially constituted by lactic acid bacteria coming from the addition of starter cultures and developed in the first production phases.

The concentration of *Pseudomonas* spp. ranged between 3,65 and 3,90 Log CFU/g at T0, then decreased, as a result of drying and acidification of salami; from T30-T40, these microorganisms were not further detected. Also, *Enterobacteriaceae*, whether naturally present in fresh pork, were almost constantly below 2 Log CFU/g. These values indicated a good hygiene of the meat used. The vacuum packaging of salami did not cause significant changes in the bacterial microflora.

The pH of the analysed salami showed a typical trend: starting from an initial value of 5.71-5.72 typical of fresh meat, an initial decline was observed in the early stages, due to the fast growth of added LAB. Then a gradual increase was observed, to 5.42-5.55, due to the normal proteolytic reactions occurred during the maturation of the product.

The same trend was observed in the vacuum packaged samples, where curing phenomena have led to a slight increase of the pH, reaching values similar to those found in the unpackaged samples and stored for the same period.

The pH values observed during the trial are not sufficient to explain the absence of growth of *Listeria* spp. in all the batches. The a_w, was 0.950-0.954 at T0, then decreased during the drying and the first part of the curing: at T20, when the product could be sold, it reached a median value of 0.920 (0.919-0.940), limit of growth for *L. monocytogenes,* if considered as a single factor. In the following steps the aw steadily

Table 1. Optical density values detected in inoculated samples (reading against blank broth, expressed as difference from the value at inoculation).

Condition of storage		*L. innocua* ATCC 33090	*L. innocua* (salami substrate)	*L. monocytogenes* 12MOB045LM	*L. monocytogenes* 12MOB085LM
37°C	24 h	1.429	1.395	1.155	1.185
	48 h	0.972	0.994	0.417	0.614
20°C	24 h	0.064	0.169	0.231	0.266
	48 h	1.364	1.446	1.333	1.304
20°C, pH 5.5	24 h	-0.003	0.045	0.063	0.053
	48 h	0.816	0.928	0.782	1.059
20°C, 3% NaCl	24 h	0.015	0.026	0.00	0.04
	48 h	1.308	1.488	1.301	1.262

Table 2. Microbiological and chemical-physical characterization of control samples of batch 1.

Parameter	T0	T4	T20	T30	T40	T50	T60	T70	T80	T90
TVC*	7.00	7.95	8.53	8.65	8.32	8.52	8.23	8.46	8.38	8.56
Pseudomonas spp.*	3.65	3.26	2.48	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
*Enterobacteriaceae**	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
LAB*	5.30	8.11	8.57	8.56	8.36	8.53	8.34	8.57	8.51	8.38
pH	5.72	5.27	5.24	5.31	5.32	5.36	5.38	5.38	5.40	5.42
aw	0.954	0.931	0.920	0.909	0.890	0.881	0.856	0.853	0.832	0.828
			T20+30	T30+30	T40+30	T50+30	T60+30	T70+30	T80+30	T90+30
TVC*	-	-	8.41	8.30	8.23	8.19	8.36	8.04	8.30	7.84
Pseudomonas spp.*	-	-	3.65	3.26	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
*Enterobacteriaceae**	-	-	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
LAB*	-	-	8.56	8.28	8.54	8.37	8.33	8.21	8.36	8.04
pH	-	-	5.35	5.36	5.40	5.48	5.53	5.56	5.56	5.51
aw	-	-	0.914	0.904	0.888	0.871	0.859	0.845	0.844	0.826

*TVC, *Pseudomonas* spp., *Enterobacteriaceae* and LAB are expressed as Log CFU/g.

Table 3. Microbiological and chemical-physical characterization of control samples of batch 2.

Parameter	T0	T4	T20	T30	T40	T50	T60	T70	T80	T90
TVC*	5.85	8.30	8.23	8.04	7.99	8.56	7.95	8.18	8.09	8.00
Pseudomonas spp.*	3.83	3.41	2.85	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
*Enterobacteriaceae**	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
LAB*	5.17	8.48	8.40	8.36	8.26	8.60	7.95	8.30	8.27	8.18
pH	5.71	5.26	5.28	5.34	5.40	5.38	5.54	5.53	5.47	5.46
aw	0.950	0.943	0.919	0.923	0.898	0.900	0.877	0.881	0.864	0.845
			T20+30	T30+30	T40+30	T50+30	T60+30	T70+30	T80+30	T90+30
TVC*	-	-	8.30	8.30	7.99	8.78	7.48	8.00	8.26	7.51
Pseudomonas spp.*	-	-	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
*Enterobacteriaceae**	-	-	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
LAB*	-	-	8.18	7.85	8.01	8.29	8.02	7.89	8.24	7.66
pH	-	-	5.42	5.42	5.47	5.47	5.54	5.61	5.62	5.55
aw	-	-	0.931	0.924	0.916	0.916	0.894	0.861	0.873	0.831

*TVC, *Pseudomonas* spp., *Enterobacteriaceae* and LAB are expressed as Log CFU/g.

Table 4. Microbiological and chemical-physical characterization of control samples of batch 3.

Parameter	T0	T4	T20	T30	T40	T50	T60	T70	T80	T90
TVC*	5.55	8.00	8.23	8.28	8.25	7.85	8.20	8.54	7.63	8.97
Pseudomonas spp.*	3.90	3.20	2.85	2.00	< 2.00	2.30	< 2.00	< 2.00	< 2.00	< 2.00
*Enterobacteriaceae**	< 2.00	2.90	< 2.00	2.30	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
LAB*	5.12	8.70	8.23	8.48	8.32	8.41	8.32	8.60	8.04	8.13
pH	5.72	5.2	5.39	5.41	5.44	5.48	5.56	5.56	5.55	5.54
aw	0.951	0.950	0.940	0.903	0.912	0.904	0.882	0.850	0.842	0.863
			T20+30	T30+30	T40+30	T50+30	T60+30	T70+30	T80+30	T90+30
TVC*	-	-	8.11	8.00	7.70	8.03	8.15	8.23	8.00	7.90
Pseudomonas spp.*	-	-	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
*Enterobacteriaceae**	-	-	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
LAB*	-	-	8.38	8.11	7.82	8.18	8.24	8.30	7.81	7.82
pH	-	-	5.44	5.51	5.57	5.53	5.65	5.58	5.57	5.55
aw	-	-	0.935	0.927	0.910	0.897	0.873	0.871	0.864	0.852

*TVC, *Pseudomonas* spp., *Enterobacteriaceae* and LAB are expressed as Log CFU/g.

decreased till T90 to 0.828-0.863, values that certainly inhibit microbial growth. As expected, a_w values were not changed by vacuum storage for 30 days.

Growth potential of *Listeria* spp. in "Bergamasco" salami

The trends of *Listeria* spp. concentration in the inoculated samples are shown in Table 5. As can be seen from the data reported, for batch 1, in the initial period, a decrease in *Listeria* spp. inoculated in the product was obtained, with subsequent stabilization. No increase in *Listeria* spp. loads was observed at any time, considering T0 or T20 starting values. The same trend was observed in the samples stored under vacuum, in which a further decrease of the concentration was generally detected during the storage at 12°C for 30 days (average decrease was 0.32 Log CFU/g from T0). The value of δ calculated for batch 1 was at T90 equal to -1.04.

In batch 2 (Table 5), a slight increase of *Listeria* spp. concentration in inoculated units was observed from T0 to T20 (+0.25 Log CFU/g), then a gradual decrease occurred during the rest of the trial (-0.65 Log CFU/g at T80). Decreasing concentrations were observed also in vacuum packaged units during the storage at 12°C for 30 days, with an average decrease of 0.42 (+0.11/-1.53) Log CFU/g. The value of δ found in batch 2 was finally of -0.59 Log CFU/g.

In batch 3, an initial stable trend of *Listeria* spp. counts, followed by a decrease in the second part of the trial (especially from T50) was detected. As for the other two batches, no increase in *Listeria* spp. loads was observed at any time, considering both the initial values and the values recorded at T20. As for the other batches, the vacuum packaging resulted in a decrease in the bacterial loads with an average reduction of 0.34 Log CFU/g (-0.09/-1.73). The value of δ found in batch 3 was -0.87.

Discussion

"Bergamasco" salami showed to be characterized by a very high microflora concentration level, typical for this product, due to the addition of starters cultures to the salami mass. These microorganisms, during the first phases of drying and curing of the product, could represent the potential competitors of *Listeria* spp., that may be present in raw meat. The mechanisms of action have been extensively studied (competition for nutrients, production of organic acids, bacteriocins, etc.). It is known that the achievement of a "critical" load of lactic bacteria causes a stop of the replication of other bacteria that may be present ("Jameson effect") (Jameson, 1962; Gálvez *et al*., 2008). In the case of the Bergamasco salami, the growth of LAB rapidly reached a plateau around 8 Log CFU/g, leading to an acidification of the mass and a substantial stabilization of the product. The pH of the "Bergamasco" salami followed the expected curves for this type of products, characterized by an acidification, caused by the rapid development of lactic acid bacteria due to the high temperatures applied during drying, while subsequently a gradual increase of the values was observed. Water activity, thanks to the initial drying phase, reached unfavourable values for *Listeria* spp. growth, and gradually decreased during curing, making the substrate less suitable for its development.

The combination of microbiological and chemical-physical factors that characterized the product has never allowed the development of *L. innocua* inoculated strains; among the factors, the greatest influence was exerted by the water activity, already completely inhibitory starting from T20-T30, combined with the high and constant loads of competing LAB; pH values acted as a further hurdle to microbial growth, although alone it was not enough to completely inhibit the growth.

Considering growth potential of *Listeria* spp., the loads measured during the test carried out on three different batches, indicated the absence of a significant growth in the product. The further storage under vacuum of salami, even in thermal abuse, did not determine any development of the inoculated *L. innocua* strains, resulting in a further decrease of about 0.3-0.4 Log CFU/g.

Considering, for each withdrawal time, the least favourable batch (thus the highest growth potential obtained among the three replicates), it was therefore evident that, both in the samples stored in curing room at a temperature of 14-16°C and a humidity of 78-80% (conditions more permissive than those used during the normal storage of the product), and in the vacuum packaged samples stored at 12°C, the threshold of +0.5 Log CFU/g, defined by the EURL Lm guidelines for the development of *L. monocytogenes* was never overcome.

In conclusion, the product tested can be classified, according to the EC Reg. 2073/2005, Annex I, in the category 1.3 as "Ready-to-eat food that is not favourable for growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes". The Regulation therefore only prescribes the need to ensure that *L. monocytogenes* loads do not exceed the

Table 5. Median growth potential values of *L. innocua* in inoculated samples maintained in the ageing room or vacuum packaged and maintained at 12°C (results reported as difference between T0 values and T20 values in Log CFU/g). In italics are indicated the less favorable data among the three batches.

LogCFU/g	Batch	T0	T4	T20	T30	T40	T50	T60	T70	T80	T90
δ T0	1	2.52*	-0.22	-0.26	-0.37	-0.33	-0.36	*-0.21*	-0.39	-1.04	-1.04
	2	2.13*	*+0.25*	*+0.20*	*+0.12*	*+0.06*	*-0.05*	*-0.23*	*-0.23*	*-0.65*	*-0.59*
	3	*2.48**	-0.24	-0.18	-0.11	-0.21	-0.63	-0.33	-0.45	-1.18	-0.87
δ T20	1	-	-	2.26**	-0.11	-0.07	*-0.10*	*+0.05*	*-0.13*	*-0.78*	-0.78
	2	-	-	2.33**	-0.07	-0.14	-0.25	-0.43	-0.43	-0.85	-0.79
	3	-	-	*2.30***	*+0.07*	*-0.03*	-0.45	-0.15	-0.28	-1.00	*-0.70*
				T20+30	T30+30	T40+30	T50+30	T60+30	T70+30	T80+30	T90+30
δ T0	1 (vac.)	-	-	-0.39	-0.92	-0.36	-0.54	*-0.52*	-1.34	*-0.98*	*-1.52*
	2 (vac.)	-	-	*-0.26*	*-0.15*	*+0.11*	*-0.48*	-0.73	*-0.59*	-1.13	-1.53
	3 (vac.)	-	-	-0.66	-0.42	-0.29	-0.52	-1.08	-1.30	-1.08	-1.78
δ T20	1 (vac.)	-	-	*-0.13*	-0.66	-0.10	*-0.28*	*-0.26*	-1.09	*-0.72*	*-1.26*
	2 (vac.)	-	-	-0.45	-0.35	*-0.09*	-0.68	-0.93	*-0.79*	-1.33	-1.73
	3 (vac.)	-	-	-0.49	*-0.24*	-0.11	-0.35	-0.90	-1.12	-0.90	-1.60

**Listeria* spp. concentration at T0; ***Listeria* spp. concentration at T20; vac. = vacuum.

level of 100 CFU/g during the commercial life, as the absence of the pathogen in the product before leaving the production plant, is not mandatory. Finally, it is important to specify that, any eventual modification of the recipe or of the production process of "Bergamasco" salami needs to be carefully evaluated, in order to keep unfavourable conditions for the growth of *L. monocytogenes* and thus to ensure the safety of this traditional meat product.

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