PE6.09 Behaviour of Listeria Monocytogenes During Shelf-Life of Sliced Italian Salami 359.00

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Abstract—The aim of this study was to evaluate the behaviour and the possible growth of L. monocytogenes in sliced PA-packed salami to provide quantitative data on the fate of Listeria in salami with pH and aw values which are usual in that kind of products during shelf-life. For the study, 4 types of salami having different production characteristics were used. The results showed that L. monocytogenes can not grow in matured salami with water activity values from 0.92 to 0.95 and pH values from 5.1 to 5.7. Inhibition was recorded both at refrigeration temperatures (4 and 8° C) and under temperature abuse conditions (15, 21 and 25°C).

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Index Terms— Listeria monocytogenes, Salami, shelf-life

I. INTRODUCTION

THE European Regulation, n. 2073/2005 come into force in January 2006 and focusing on the microbiological criteria to be applied to food products, approaches the Listeria issue in a new way, indicating tolerance criteria for ready-to-eat products [10]. The core of that approach is the consideration that the presence of the pathogen in some categories of foods intended for being consumed without any further listericide treatment (the RTE class), is in contrast with the relatively low incidence of the recorded cases of listeriosis. Risk assessment studies have indicated that low levels of L. monocytogenes in food products correspond to a low risk for the consumer's health [5, 11]. The applicability of tolerance criteria for L. monocytogenes in RTE food products is defined according to a distinction based on physico-chemical parameters between products in which Listeria growth is possible and those where it is not. Products with pH ≤ 4.4 or aw ≤ 0.92, products with pH ≤ 5.0 and aw ≤ 0.94, products with a shelf-life lower than 5 days are automatically included in the second group and therefore the presence of L. monocytogenes in those products will be allowed at levels ≤ 100 cfu/g. According to those indications, some types of Italian salami would be excluded from the category of products where that tolerance exists, provided that, to the satisfaction of the competent authority, during the shelf-life the established limit is not overcome [2]. The aim of this study was to evaluate the behaviour and the possibility of growth of L. monocytogenes in Protective Atmosphere (PA) packed sliced salami during shelf-life.

II. MATERIALS AND METHODS

The selection of the products on which the Microbial Challenge Test (MCT) should be performed was made according to a criterion of variety of the physico-chemical parameters so as to be able to identify products that were as representative as possible of the types present on the market (Table 1).

A. Physico – chemical analyses pH: measured by probe insertion into the finely minced sample (pHmeter WTW mod. pH330i, equipped with Double pore electrode, Hamilton, CH). Water activity: AquaLab® (Model Series 3TE, Decagon Devices, Inc.).

B. Experimental design of the Microbial Challenge Test (MCT) for Listeria monocytogenes MCT was designed and performed according to the guidelines developed by Scott et al. [12] and approved by FSIS/USDA in 2006 [3]. For each of the four types of salami investigated the same procedure was applied. The product was sliced with a bench slicer and placed into PP/EVOH/PP trays (9.5 cm x 14.5 cm x 1.8 cm) (25 g each). For the inoculation a cocktail of five strains of L. monocytogenes (L.m. Scott A and L.m. ATCC 7644, L.m. 313, L.m. 223 and L.m. 221, SSICA collection) was used. Inoculum suspension was confirmed, cultured, prepared and

stored as described in a previous work [6]. Packing was performed by using a semi-automated packing machine Food Basic V/G (I.L.P.R.A.) replaced, on sealing the trays, the headspace atmosphere by a PA consisting of 30% CO2 and 70% N2. For tray sealing a transparent film made of PET/PP/EVOH/PP (PET = polyethyleneterephthalate) was used.

C. Microbiological analyses Microbiological analyses were performed at 0, 30, 60 e 90 days for each storage temperature. At pre-established times 3 to 5 packs were sampled for each incubation temperature (4, 8, 15, 21 and 25 °C) and Listeria counting was performed on the entire content of the pack. Samples of whole and pre-sliced salami were analysed for: Aerobic Plate Count (APC), Enterobacteriaceae, Lactic acid bacteria, Micrococcus and Staphylococcus, Brochothrix thermosphacta, Staphylococcus aureus, Listeria monocytogenes [6].

D. Analysis of results The results were transferred into a log scale for normalization of distribution. The means were compared by analysis of variance with one grading criterion to evaluate whether statistically significant differences were present or not. In Listeria quantitative analysis, in case a result is lower than the analytical detection limit (< 3 cfu/g), data processing was performed by considering half threshold value (1.5 cfu/g equivalent to 0.18 log cfu/g and 1.57 log cfu/package.

III. RESULTS AND DISCUSSION

The physico-chemical and microbiological characteristics of the salami under investigation are reported in Table 2. The data confirm that the four types of salami don't belong to the food category "substrate unable to support the growth of L. monocytogenes " and therefore appropriate for setting up the MCT.

Microbiological profiles found are closely related to the typical microbiota of "Italian salami", i.e. a high microbial load, higher than 108 cfu/g, consisting of lactic acid bacteria followed by the staphylococci and micrococci group, at levels ranging from 105 and 107 cfu/g, depending on the type of salami analysed [1, 4]. The results of the four MCTs, one for each type of salami, are shown in Table 3. Listeria inocula were 39, 110, 118 and 1300 cfu/g, respectively. From a first evaluation it comes out that no increase in the initial Listeria values was ever recorded in any combinations among types of salami, time and temperature. The observation of average data and relative standard deviations shows a steady reduction in the initial contamination level at all temperatures with a more marked inactivation at 21 e 25°C (temperature abuse). In type 1 salami, which was contaminated with less than 100 cfu/g of Listeria to reproduce an authentic level of post-processing contamination, the analytical detection limit was already achieved after 30 days of storage at 21 and 25°C. At 4, 8 and 15°C that limit is attained on day 90.

In MCTs performed in salami types 2 and 3 an initial contamination of around 100 cfu/g was selected to assess the limit set by the Regulation; in that case too, no growth was recorded. On the contrary, the minimum detection limit was achieved after 30 and 60 days of incubation at 25 and 21°C, respectively. In type 4 salami the high inoculation level allowed obtaining a count which was less affected by the detection limit and therefore a better observation at high temperatures of the reduction kinetics for Listeria population.

The pathogen behaviour was similar to that observed in the other MCTs. Counting data show Listeria inactivation at all temperatures, particularly at the highest ones. On day 90, qualitative analysis, carried out to determine the presence/absence of Listeria in 25 g, indicated absence in three out of three samples stored at 21 e 25°C; analysis on samples at 15°C revealed its presence in one out of the three samples analysed. The results obtained in the various types of salami confirmed the marked inactivation occurring during storage at temperatures higher than 15° C, according to what already found by other authors, in aged meat products [6 - 9].

IV. CONCLUSION

Matured salami is a complex ecosystem where the interaction between the different "hurdles", more or less known, determines the inhibition of Listeria growth. The results reported in this study showed that there exists no possibility of Listeria growing in matured salami with values of aw from 0.92 to 0.95 and pH from 5.1 to 5.7 which, according to predictive microbiology, should instead favour the growth. Inhibition was recorded both at refrigeration temperatures (4 and 8° C) and under temperature abuse conditions (15, 21 and 25°C). A

comparison made between the results obtained by applying predictive models and the actual ones obtained by the Microbial Challenge Testing carried out on four different types of salami, showed that the prediction model was unable to correctly describe the behaviour of Listeria in such a complex matrix as salami.

ACKNOWLEDGEMENT

The authors thank Mrs. Gloria Attolini and Mr. Turno Pedrelli for contributing their time, talent and opinion to this work.

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Table 1
Types and characteristics of the salami under investigation

Characteristics	**Types of Salami**			
	1	2	3	4
Casing	Cloth	Cloth	Collagenic	Natural
Diameter	15*	10	10	6
Granulometry	8	8	3.5	7
Fat %	20	20	32 - 34	28 - 30
Average weight of the product	5	3	3	1

* ovoidal

Table 2
Microbiological (log cfu/g) and physico-chemical characterization of salami

Tests	**Types of Salami**			
	1	2	3	4
Aerobic plate count	8.67	8.30	8.52	8.99
Lactic acid bacteria	8.63	8.30	8.23	8.96
Micrococcus and Staphylococcus	7.30	7.51	5.91	6.73
Enterobacteriaceae	1.76	2.60	1.08	2.45
Brochothrix thermosphacta	3.38	1.72	1.95	1.48
Staphylococcus aureus	0.48	2.49	1.93	2.41
Water activity	0.945	0,943	0,945	0.925
pH	5.2	5.1	5.3	5.7

Table 3
Fate of *L. monocytogenes* during shelf-life - log cfu/package ± St. dev.- average of three determinations

Temperature °C	Days	Types of Salami			
		1	2	3	4
	0 (4 hours)	2.99 ± 0.00	3.44 ± 0.10	3.47 ± 0.31	4.5 ± 0.12
4	30	2.14 ± 0.37	3.36 ± 0.15	3.21 ± 0.11	3.99 ± 0.1
	60	1.75 ± 0.27	2.75 ± 0.14	2.77 ± 0.18	3.55 ± 0.2
	90	1.57 ± 0.00	2.53 ± 0.21	2.37 ± 0.36	2.95 ± 0.3
8	30	2.18 ± 0.25	3.10 ± 0.10	3.26 ± 0.06	3.72 ± 0.0
	60	1.65 ± 0.15	2.51 ± 0.23	2.48 ± 0.44	3.43 ± 0.2
	90	1.57 ± 0.00	2.09 ± 0.30	2.07 ± 0.26	2.57 ± 0.4
15	30	1.88 ± 0.30	2.87 ± 0.04	3.23 ± 0.12	3.42 ± 0.0
	60	1.67 ± 0.17	1.97 ± 0.30	2.23 ± 0.39	2.38 ± 0.0
	90	1.57 ± 0.00	1.57 ± 0.00	1.80 ± 0.29	1.57 ± 0.0
21	30	1.67 ± 0.17	1.77 ± 0.17	2.59 ± 0.62	2.83 ± 0.2
	60	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.0
	90	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.0
25	30	1.57 ± 0.00	1.57 ± 0.00	1.67 ± 0.17	1.88 ± 0.0
	60	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.00	1.65 ± 0.1
	90	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.00	1.57 ± 0.0