Brazilian Journal of Microbiology (2008) 39:514-516
ISSN 1517-8382

BEHAVIOUR OF *L. MONOCYTOGENES* IN SLICED, VACUUM-PACKED MORTADELLA

Luciano dos Santos Bersot[1]*; Cíntia Gillio[1]; Paula Tavolaro[1]; Mariza Landgraf[1]; Bernadette Dora Gombossy de Melo Franco[1]; Maria Teresa Destro[1]

[1]Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, Brasil.

Submitted: October 11, 2006; Returned to authors for corrections: April 20, 2007; Approved: August 11, 2008.

ABSTRACT

This study evaluated the growth of naturally occurring *L. monocytogenes* in sliced, vacuum-packed mortadella samples during storage at 5ºC until the expiration date. Tukey's test indicated that counts of *L. monocytogenes* on 0, 10, 20, 30 and 40 days of storage were significantly different ($p<0.05$), indicating growth during shelf life. In three trials, the mean increase was 1.72 log cycles. Vacuum packing and storage under refrigeration were not effective in controlling the growth of *L. monocytogenes* in sliced mortadella, indicating that good manufacturing practices and implemented HACCP programs are essential to assure safety of this product.

Key-words: *Listeria monocytogenes*; growth; sliced mortadella; vacuum-packing

Listeria monocytogenes is ubiquitous and almost all kinds of foods may be contaminated with this bacterium. Equipment and utensils used in the food industry are considered important sources of contamination, and it is generally accepted that total elimination of *L. monocytogenes* from all foods is impracticable and may even be impossible. An important characteristic of *L. monocytogenes* is the capability to survive and grow under refrigeration, which is usually an obstacle for the growth of most foodborne pathogens. This fact is extremely relevant, mainly when ready-to-eat (RTE) products are considered, as they may have not been submitted to a listericidal step during processing and/or may have been contaminated after processing. When present, growth of *Listeria* may be favored by refrigeration in detriment of other microorganisms (10,12,13)

Epidemiological evidences indicate that food products that present the greatest risk for listeriosis dissemination are those that support the growth of the pathogen, have long shelf-life under refrigeration and are ready-to-eat (9,18).

In general, contamination of meat products occurs as primary contamination of the meat but also via ingredients and environment (3). A previous study has shown that *L. monocytogenes* does not survive the cooking process of mortadella (1) indicating that presence of the pathogen in vacuum-packed sliced product is due to cross-contamination during slicing/packaging.

Information on *L. monocytogenes* behavior in RTE products is important for the development of new strategies to control this pathogen in the product. Thus, the objective of the present study was to evaluate the capability of naturally occurring *L. monocytogenes* to grow in sliced, vacuum-packed mortadella stored under refrigeration.

Sliced, vacuum packed mortadella samples were obtained from a meat industry in packs of 250 g on the day of packaging. This industry received the mortadella in pieces of 5 kg in the original casing from another meat plant and sliced and packed the product using a fully automatized procedure. According to the label, the shelf life of the sliced product was 40 days under refrigeration, without specifying the temperature. The samples were placed in isothermal containers, transported to the laboratory and were tested immediately (day zero) or stored at 5ºC.

Quantification of *L. monocytogenes* was done on days zero, 10, 20, 30 and 40, using the method described by Farber *et al.* (6) in a Most Probable Number technique format (14). In each sampling day, three packs of 250 g were analyzed. Fifty grams of mortadella were asseptically removed from each package and

*Corresponding Author. Mailing address: UFPR, Campus Palotina, Rua Pioneiro, 2153, Jardim Dallas, Palotina, PR, 85.950-000, Palotina, PR, Brasil. Fax: (+5544) 3649-6616. E-mail: lucianobersot@ufpr.br

transferred to plastic bags containing 450 mL of Listeria Enrichment Broth, UVM Formulation (LEB) (Oxoid, England). After homogenization, the mixture was submitted to four decimal dilutions (equivalent to 10g, 1g, 0.1g and 0.01g of product) in LEB, in triplicates, and incubated at 30ºC for 24h. For identification of *L. monocytogenes*, the methodology of Farber et al. (6) was carried out. The experiments were run three times (trials A, B and C).

Results were submitted to variance analysis and Tukey's test, using Statistic for Windows, release 5.0 (Stat Soft Inc, 1984-1995).

Table 1 shows the mean populations of *L. monocytogenes* ($MPNg^{-1}$) in the sliced, vacuum-packed mortadella samples, in the three trials, and the mean counts for each of the five days of analysis (days 0, 10, 20, 30 and 40). Final populations at the end of the shelf life were 89.1, 23.4 and 102.3 $MPN.g^{-1}$ for trials A, B and C, respectively. Variance analysis of the results indicated that differences in these populations were not significant ($p>0.05$). Tukey's test, applied to the general means, showed that differences in populations on day 10 compared to day 0, on day 20 compared to day 10, on day 20 compared to day 30 and on day 40 compared to day 30 were significant ($p < 0.05$), indicating multiplication of *L. monocytogenes* during the shelf life of the product stored under refrigeration (5ºC).

Similar populations of *L. monocytogenes* at the end of shelf life were reported elsewhere. Examining sliced vacuum-packed frankfurters the authors found counts around 100 – 200 CFU. g^{-1} of the product (16,17).

It is interesting to note that in two out of three trials the population of *L. monocytogenes* on the 30th day of storage was lower than that observed on the 20th day. This decrease in *L. monocytogenes* population may be explained by the presence of autoctonous lactic acid bacteria (LAB), well-known for their capability of inhibiting growth of *L. monocytogenes* during food storage (4). Contamination with LAB can occur during slicing and packaging procedures, mainly when hygiene conditions are precarious.

Table 2 shows that *L. monocytogenes* population increase (mean $MPN.g^{-1}$) during storage at 5ºC for 40 days was equal to 76.4, 22.1 and 89.0 for trials A, B and C, respectively. General mean increase of *L. monocytogenes* was 62.5 MPN. g^{-1}. According to several reports, *L. monocytogenes* population can increase up to 6 logs in vacuum-packed meat products stored under refrigeration (3,7,8,11,17). However these studies were conducted with spiked products and the conditions required for the growth of microorganisms are not necessarily the same as for naturally contaminated foodstuffs. In artificially contaminated products, the microbial cells are in optimal physiological conditions, favoring the competition with the normal microbiota of the product.

Refrigeration between 4 and 8ºC of sliced, vacuum-packed meat products does not seem to be an obstacle to *L. monocytogenes* growth, as observed in the present study and also by Rosso *et al*. (15). Temperatures below 2ºC are recommended for the control of *L. monocytogenes* in these products; however, these temperatures are not commonly achieved at retail level. It should be also taken into account that *L. monocytogenes* tolerates low O_2 tension, being able to survive and grow in anaerobic conditions, even at low temperatures (13), as encountered in vacuum-packed mortadella stored under refrigeration.

The low initial *L. monocytogenes* population in the products found in this study has also been observed by other authors (5,16,17). However, these low initial numbers are not an assurance of low risk for the product since the pathogen may grow and reach risky levels ($\geq 100 g^{-1}$) (13).

The method employed for enumeration of *L. monocytogenes* in a food product has strong influence in the results (2). Once

Table 1. Mean populations of *L. monocytogenes* in sliced, vacuum-packed mortadella, during storage under refrigeration for 40 days.

	Mean population ($MPN.g^{-1}$)*			General mean population**
Day	Trial A	Trial B	Trial C	($MPN.g^{-1}$)
0	0.03	1.00	0.25	0.40^{a***}
10	0.14	0.72	11.20	4.00^{a}
20	95.50	0.50	41.70	45.90^{b}
30	12.60	1.60	2.40	5.50^{a}
40	89.10	23.40	102.30	71.6^{b}

* mean of three determinations

**mean of trials A, B and C

***same letters indicate values that are statistically similar ($p>0.05$)

Table 2. Increment in population of *L. monocytogenes* in sliced, vacuum-packed mortadella during storage under refrigeration for 40 days

Interval in days	Increment ($MPN.g^{-1}$)*			General increment
	Trial A	Trial B	Trial C	($MPN.g^{-1}$)
0 – 10	0.10	-0.28	10.95	3.60
10 – 20	95.4	-0.22	30.50	41.9
20 – 30	-82.9	1.10	-39.3	-40.4
30 – 40	76.5	21.8	99.9	66.1
Total increment	76.4	22.1	89.0	62.5

*mean of three determinations.

initial population of the pathogen can be as low as 0.03 MPN g^{-1} (Table 1), one may not find *L. monocytogenes* in the product if the method lacks sensitivity.

In the present study *L. monocytogenes* was found in all sliced mortadella samples. This result differs from the one observed in a previous study (1), carried out with non-sliced mortadella, where the pathogen was detected in 26.7% of the samples. These findings indicate that slicing has an important role in the contamination process.

In view of the fact that *L. monocytogenes* can reach risky levels in vacuum-packed sliced mortadella stored under refrigeration, appropriate control measures are needed to minimize contamination of the product and/or inhibit the growth of the pathogen. Implementation of good hygiene practices (GHP), SSOPs and good manufacturing practices (GMP), together with HACCP is of uppermost importance to minimize environmental and product contamination. Reduction of shelf life, reformulation of the product by adding inhibitory ingredients, post-packing listericidal treatment (e.g. irradiation or ultra-high pressure) and active packaging are some options for the control growth of the pathogen in sliced meat products such as mortadella.

ACKNOWLEDGEMENTS

The authors would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP, for first author's fellowship and for project grant.

RESUMO

Comportamento de *L. monocytogenes* em mortadelas fatiadas e embaladas a vácuo

O presente trabalho avaliou a multiplicação de *L. monocytogenes* naturalmente presente em mortadelas fatiadas, embaladas a vácuo e estocadas a 5ºC durante sua vida de prateleira. O teste Tukey indicou que as populações de *L. monocytogenes* nos tempos 10, 20, 30 e 40 dias diferiram significativamente ($p<0,05$) indicando multiplicação durante o armazenamento. Em três repetições, o aumento médio foi de 1,80 ciclos log. A embalagem a vácuo e estocagem sob refrigeração não foram suficientes para o controle da multiplicação de *L. monocytogenes* em mortadelas fatiadas, indicando que as boas práticas de fabricação e um sistema HACCP implantado são fundamentais para assegurar a segurança desse produto.

Palavras-chave: *Listeria monocytogenes*; mortadela fatiada; embalagem a vácuo.

REFERENCES

1. Bersot, L.S.; Landgraf, M.; Franco, B.D.G.M.; Destro, M.T. (2001). Production of mortadella: behavior of *Listeria monocytogenes* during process and storage conditions. *Meat Sci.*, 57: 13-17.
2. Donnelly, C.W.; Nyachuba, D.G. (2006). Conventional methods for detection of *Listeria*. *In:* Ryser, E.T., Marth, E.H. (eds) Listeria, Listeriosis and Food Safety CRC Press, p. 215-256.
3. Duffy, L.L.; Vanderlinde, P.B.; Grau, F.H. (1994). Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, a_w, nitrite and ascorbate. *Int. J. Food Microbiol.*, 23: 377-390.
4. Farber, J.; Pagotto, F.; Scherf, C. (2006). Incidence and behavior of *Listeria* monocytogenes in meat products. *In:* Ryser, E.T., Marth, E.H. (eds) Listeria, Listeriosis and Food Safety CRC Press, p. 503-570.
5. Farber, J.M.; Daley, E. (1994). Presence and growth of *Listeria monocytogenes* in naturally-contaminated meats. *Int. J. Food Microbiol.*, 22: 33-42.
6. Farber, J.M.; Warburton, D.W.; Babiuk, T. (1994). Isolation of *Listeria monocytogenes* from all food and environmental samples. *In*: Canada. Health and Welfare. *Compendium of analytical methods*: laboratory procedures of microbiological analysis of foods. Ottawa, *Polyscience*, v. 3, 16 p. [MFHPB30].
7. Glass, K.A.; Doyle, M.P. (1989). Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environment. Microbiol.*, 55: 1565-1569.
8. Guang-Hua, W.; Mao-Zhan, S. (1997). The behaviour of *Listeria monocytogenes* in vacuum-packed sliced chinese spiced beef. *Fleischwirtsch*., 77: 57-58.
9. ILSI Research Foudantion (2005). Achieving continuous improvement in reductions in foodborne listeriosis – a risk-based approach. *J. Food Prot.*, 68: 1932-1994.
10. Kornacki, J.L.; Gurtler, J. (2006). Incidence and control of *Listeria* in food processing facilities. *In:* Riemann, H.P., Cliver, D.O. (eds) Foodborne Infections and Intoxications. *Academic Press*, p. 681-766.
11. Krämer, K.H.; Baumgart, J. (1993). Sliced frankfurter-type sausage: inhibiting *Listeria monocytogenes* by means of a modified atmosphere. *Fleischwirtsch.*, 73: 1279-1280.
12. McCarthy, S.A. (1997). Incidence and survival of *Listeria monocytogenes* in ready-to-eat seafood products. *J. Food Prot.*, 60: 372-376.
13. Pagotto, F.; Corneau, N.; Farber, J. (2006). *Listeria monocytogenes* infections. In: Riemann, H.P., Cliver, D.O. (eds) Foodborne Infections and Intoxications. *Academic Press*, p. 313-340.
14. Peeler, J.T.; Houghtby, G.A.; Rainosek, A.P. (1992). The most probable number technique. *In*: Vanderzant, C., Splittstoesser, D.F. *Compendium of methods for the microbiological examination of foods.* (pp. 105-120). Washington: APHA.
15. Rosso, L.; Bajard, S.; Flandrois, J.P.; Lahellec, C.; Fournaud, J.; Veit, P. (1996). Differential growth of *Listeria monocytogenes* at 4 and 8 degrees: consequences for the shelf life of chilled products. *J. Food Prot.*, 59: 944-949.
16. Schmidt, U. (1995). Sliced, vacuum-packed frankfurter-type sausage: technological measures to inhibit the growth of listeriae. *Fleischwirtsch.*, 75: 804-807.
17. Schmidt, U.; Kaya, M. (1990). Behaviour of *L. monocytogenes* in vacuum-packed sliced frankfurter-type sausage. *Fleischwirtsch.*, 70: 1294-1295.
18. Swaminathan, B.; Cabanes, D.; Zhang, W.; Cossart. P. (2007). *Listeria monocytogenes*. *In:* Doyle, M.P., Beuchat, L.R. (eds) Food Microbiology: fundamental and frontiers. 3. ed. Washington, D.C.: ASM Press, p. 322-341.