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Behavior of *Listeria monocytogenes* type1 355/98 (85) in meat emulsions as affected by temperature, pH, water activity, fat and microbial preservatives

Pellicer Karina [a,*], Copes Julio [a], Giannuzzi Leda [b], Zaritzky Noemi [b,c]

[a] Cátedra de Tecnología e Higiene de los Alimentos, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, 60 y 118, 1900 CC 296 La Plata, Argentina
[b] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CONICET, UNLP 47 y 116, CP 1900 La Plata, Argentina
[c] Facultad de Ingeniería, Universidad Nacional de La Plata, 1 y 47, CP 1900 La Plata, Argentina

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ABSTRACT

The aim of this work was to analyze the effect of temperature (10–30 °C), fat content (20–50%), sodium chloride (2.5–5.0%) and preservative concentrations: sodium nitrite (0–150 ppm), lactic acid (50–500 mM) and nisin (0–100 IU/g (international units per gram)) on the growth of *Listeria monocytogenes* in a meat emulsion system.

Individual and simultaneous effects of the parameters were tested and the results were mathematical modeled; inhibition indexes were calculated in each case. The addition of 7.5% NaCl inhibited the growth of *L. monocytogenes* at 20 and 30 °C, however, at 10 °C, microbial counts reached approximately 10^6 CFU/g. The addition of 50 mM of lactic acid to obtain a pH $\leq$ 5 inhibited the growth of *L. monocytogenes*. The combinations of lactic acid with sodium nitrite or with nisin showed an enhancement of the inhibitory effect. However, considering the low toxicity of nisin, the combination of lactic acid (50 mM) and nisin (20 IU/g) would be more acceptable in the prevention of the growth of *Listeria monocytogenes*.

1. Introduction

Listeria monocytogenes is a pathogen which causes miscarriages and mastitis in animals whereas in human beings, it can produce infections potentially fatal in susceptible individuals. This bacterium can cause miscarriages in pregnant women and meningitis in newborns, children and adults who are immunosuppressed (Donelly, 1994; Posfay-Barbe & Wald, 2004; Seeliger, 1961; Shih-Yu et al., 2007; Smith et al., 2009). *L. monocytogenes* and *Listeria innocua* are the most frequently isolated strains in food processing plants. However, most of the cases of listeriosis transmitted through food are due to the contamination of raw or cooked foods with *L. monocytogenes* (Altekruse, Cohen, & Swerdlow, 1997; Cox et al., 1989, WHO 1988). Listeriosis has become a serious public health problem considering that, although it presents low mortality rates depending on the susceptibility of the host, its mortality is high in inmunosupressed persons (20–30%) and has a long incubation period (Chhabra, Carter, Linton, & Cousin, 1999; Donelly, 1994). The minimum infective dose of *L. monocytogenes* has not been established yet (FDA Bad Bug Book, 2009) although it has been indicated that the intake of up to 100 cells does not affect the health of healthy consumers (Jay, 1994).

L. monocytogenes can develop at low temperatures in a wide pH range, with a high concentration of sodium chloride (NaCl) and reduced water activity. Hence, it can survive and multiply in a great variety of food products (Begot, Lebert, & Lebert, 1997; Bergey's, 1986; Cole, Jones, & Holyoak, 1990; Seman, Borger, Meyer, Hall, & Milkowski, 2002). *Listeria* has been involved in a large number of food-borne outbreaks transmitted through food all over the world. The most hazardous products are those "ready to eat" which are stored at room temperature for long periods of time. *Listeria* spp. has been found in a great variety of meats and meat products, even in sausages (Ingham, Beuge, Dropp, & Losinski, 2004; Jonson, Doyle, & Cassens, 1990).

Beef sausage has a limit in fat composition; that cannot exceed the 50% of the mass of the finished product. Lactic acid without any restrictions for use, and sodium nitrite are in the group of permitted products. According to the effective regulations the use of sodium nitrite, potassium nitrate or its combination must not exceed 200 ppm (0.2 mg/g) expressed as sodium nitrite in the final product (USDA-FSIS, 1999, pp. 72185–72186 (Chapter III)).

Fermented sausage is partially dehydrated to favor its preservation for a long period of time. This product reaches pH values ranging between 6.0 and 5.1 and water activities lower than 0.94

* Corresponding author. Tel.: +54 (0)221 4236663x432; fax: +54 (0)221 4257980.
E-mail addresses: pellicerk@fcv.unlp.edu.ar (P. Karina), jcopes@fcv.unlp.edu.ar (C. Julio), leda@biol.unlp.edu.ar (G. Leda), zaritzky@ing.unlp.edu.ar (Z. Noemi).

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due to the fermentation and drying processes (Frey, 1995) therefore it does not need to be preserved at refrigeration temperature (Girard, 1991). A large number of strains of Listeria have been isolated from dry sausages (Pellicer et al., 2002).

In fermented foods, preservation is achieved through the combined effects of lactic acid and sodium chloride. During fermentation, pH first falls from 5.8 to 5.4 or 5.3 and then it increases to 5.5–5.6. The lactic acid concentration in the final product varies according to the nature and quantity of sugars in the initial product, which in dry sausages can reach average values of 250 mM lactic acid at the beginning up to 600 mM lactic acid at the end of the fermentation (Girard, 1991). During fermentation and drying of sausage, *L. monocytogenes* tends to decrease substantially, however, this organism, which is ubiquitous, psychrotrophic and relatively resistant to curing ingredients, may survive (Ingham et al., 2004; Jonson et al., 1990; Juntilla, Him, Hill, & Nurmi, 1989).

Among the natural preservatives, lactic acid can be usually found in beef, specifically in the muscle as a consequence of the glycogen metabolism, in amounts which depend on its functional condition (0.01–0.02% in the muscle at rest, approximately 0.4% in the fatigued muscle and up to 1% after cadaveric rigidity appears) (Pellegrini, Silvestre, Ochoa La Puente, & Y Ochoa La Puente, 1986; Pränd, Fischer, Schmidhoger, & Y Hans-Jurgen, 1994).

It has been reported that the undissociated lactic acid inhibits the growth of microorganisms and is frequently used to extend the shelf life of foods (Barbuddhe, Malik, & Bhilegaonkar, 1999). Lactic acid is effective to inhibit the development of *L. monocytogenes* when it reaches pH values of 4.5 and 4.6 (Ariyapitipun, Mustapha, & Clarke, 2000; Sorrels, Engil, & Hatfield, 1989).

Curing salts, such as sodium nitrite, which is currently questioned due to the hazard of nitrosamine formation, inhibits *Clostridium botulinum* (Girard, 1991). Experimental data indicate that nitrites reduce the survival time of *L. monocytogenes* and also reduce its growth rate (Buchanan, Golden, Whiting, & Smith, 1994; Buchanan, Stahl, & Whiting, 1989; Whiting & Masana, 1994).

Bacteriocins are increasingly used to control the growth of *L. monocytogenes*; among them, nisin is an antimicrobial agent that can be used in meat products (Brandt et al., 2010; Breukink & Krujiff, 1999), in combination with pH reduction and the addition of NaCl (Bouttefroy, Mansour, Linder, & Millière, 2000).

Nisin is an antimicrobial peptide which acts against *L. monocytogenes* as well as other Gram-positive bacteria. Its use is permitted in dairy products (FAO Nutrition Meeting Report Series N° 45 A (1969); Directive 95/2/CE ratified by the Commission of European Communities FAO/WHO, 2007; FDA 2006). Although nisin still does not have approval for its application in meat products in South America, some countries such as Australia and New Zealand are trying to use it, proposing the inclusion of a limit for the use of nisin in meat products (Food Standards Agency of Australia and New Zealand; October 2007 (A565)). Its potential application is high, especially to avoid the deterioration caused by Gram-positive bacteria in processed meat such as sausage and meat paste in which the homogenization of the product allows the better distribution of nisin (Ariyapitipun et al., 2000; FDA 1988; Gonalves & Massaguer, 1999). The recommended concentration is up to 400 IU/g (international units per gram) of food, which represents 10 ppm.

The aims of this work were: (a) to analyze the effect of temperature (10–30 °C), fat content (20–50%), sodium chloride (2.5–5.0%) and preservative concentrations: sodium nitrite (0–150 ppm), lactic acid (50–500 mM) and nisin (0–100 IU/g) applied both individually and in combination, on the growth of *Listeria monocytogenes* in a meat emulsion representing a model system of sausage at different storage times and (b) to model mathematically the *L. monocytogenes* counts as a function of time evaluating the Inhibition Index under the different tested conditions.

2. Materials and methods

2.1. Sample preparation

Meat emulsions were prepared as sausage model systems, with fresh lean beef and bovine fat (20, 35 and 50%) with the addition of NaCl (2.5, 5.0 and 7.5%) (Anedra).

The emulsion was prepared by using a food processor (Rowenta Universo KA 900 8750/83); water activity was evaluated using Aqualab equipment. Different concentrations of preservatives were added according to each experiment: 75,150 ppm sodium nitrite (Anedra); 50, 100, 250 and 500 mM lactic acid (Anedra) which produced pH values ranging from 5.20 to 3.22 and 10, 20, 50 and 100 IU/g nisin (Nisaplin®, Danisco UK Ltd., Dorset, UK. given by the company AMG S.R.L Argentina).

Meat emulsion samples were packed in thermoresistant polyethylene bags (85 microns thickness) and were subjected to a thermal process (100 °C, 30 min) in order to reduce the microbial load originating from the different raw materials; this procedure enabled their inoculation with the selected strain of *L. monocytogenes*, assuring that the obtained counts, were due to the growth of the inoculated strain and not due to a microorganism from the different ingredients.

Samples were stored at different temperatures (10, 20, 30 °C).

2.2. Inoculum

Reference strain of *L. monocytogenes* type 1 355/98 (85) donated by Dr. N. Leardini of the INEI/ANLIS Institute Dr. Carlos G. Malbrán was grown in brain heart infusion broth (BHI) (Oxoid) for 18 h at 37 °C with agitation from which a microbial concentration of approximately 10^8 CFU/ml was obtained. Then 1 ml of that culture was diluted in 100 ml of BHI to achieve a count of 10^6 CFU/ml. From that dilution 1 ml of 10^6 CFU/ml was inoculated in 100 g of the meat emulsion in order to obtain an initial inoculum of 10^4 CFU/g in the preparation.

The inoculated emulsions were sub-divided under sterile conditions into portions of 10 g each and packed in sterile polyethylene films.

In all the cases the experiments were carried out in triplicates, with a maximum storage time of 16 days.

2.3. Experimental design

Experiments were carried out to analyze the effect of different factors on the growth and decline of *L. monocytogenes* in meat emulsions.

2.3.1. Effect of the addition of different fat percentages (experiment A)

Meat emulsions containing 20, 35 or 50% bovine fat, 2.5% NaCl, were inoculated with *L. monocytogenes* and stored at 20 °C (Total: 3 experiments in triplicates).

2.3.2. Effect of the addition of NaCl and the storage temperature (experiment B)

The effects of storage temperature and water activity (a_w) were analyzed using a *L. monocytogenes* inoculated meat emulsion with 20% fat, and applying a 3 × 3 factorial study, with three storage temperature levels (10, 20 and 30 °C) combined with 3 different NaCl concentrations 2.5, 5.0 and 7.5% resulting to a_w values of 0.985, 0.966 and 0.955 respectively (Total: 9 experiments in triplicates).