EFFECT OF LACTATE AND DIACETATE SALTS AND HIGH PRESSURE PROCESSING ON THE SURVIVAL OF LISTERIA MONOCYTOGENES IN CURED BEEF CARPACCIO

Yanina X. Barrio[1,2]*, Diego Wilches Perez[3], Sergio R. Vaudagna[1,2,4] and Jordi Rovira[3]

1 Instituto Tecnología de Alimentos, CIA, INTA, Morón, Buenos Aires, Argentina

2 Facultad de Ingeniería y Ciencias Exactas, UADE, Buenos Aires, Argentina

3 Departamento de Biotecnología y Ciencia de los Alimentos, Universidad de Burgos, Burgos, España

4 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

*ybarrio@cnia.inta.gov.ar

Abstract – The behavior of *Listeria monocytogenes* strains and spoilage microflora, in response to the addition of antimicrobial additives (potassium lactate 3.0% or potassium lactate 1.7% and sodium diacetate 0.12%) and high pressure processing (HPP), was evaluated on cured beef *carpaccio* during refrigerated storage. Samples with or without antimicrobials were inoculated with a five-strain pool of *L. monocytogenes* (at *ca.* 10^4 cfu/g), frozen at -60°C and pressurized at 600 MPa for 5 min at room temperature. Afterwards, samples were stored at 4°C for up to 21 days. HPP alone was sufficient to reduce the *L. monocytogenes* count below the detection limit (<2 log cfu/g). Concerning antimicrobials addition, potassium lactate and sodium diacetate contributed to delay the growth of lactic acid bacteria and psycrothophs of pressurized samples during refrigeration storage.

I. INTRODUCTION

The commercialization of a wide variety of "ready-to-eat" (RTE) meat products, including fresh marinated or cured meats has been increasing in the international and local markets. Beef *carpaccio* is a traditional Italian dish prepared as thin slices from frozen raw or cured beef, which are packed under vacuum or modified atmospheres, and kept at refrigeration temperature until consumption. Because of the minimal process that *carpaccio* is subjected, and the possibility of contamination with pathogenic bacteria, such as *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes*, *carpaccio* is considered a high-risk food (1). In addition, conventional preservation technologies like thermal pasteurization cannot be applied because of the detrimental effects on product's appearance and sensory characteristics. In this sense, the use of a non-thermal technology such as High Pressure could be an alternative for *carpaccio* pasteurization. High Pressure Processing (HPP) has been successfully applied for the pasteurization of dried or cooked meat products (2). However, HPP of fresh pigmented meats causes a significant discoloration at pressure levels required for vegetative cells inactivation (>300 MPa) (3). Some studies (4, 5) have reported that HPP applied to frozen *carpaccio* improved the appearance of pressurized red meats but these treatments resulted less effective to microbial inactivation. HPP combined with natural antimicrobials has been proved in some foodstuffs (6). In this concern, lactate salts have been widely used by meat industry to extend shelf life of products. Also, some studies have shown a synergistic effect of lactate and diacetate combination against several foodborne pathogens (7).

The aim of this study was to evaluate the combined effect of lactate and diacetate salts and HPP on the survival of *Listeria monocytogenes* and spoilage microflora of cured beef *carpaccio*.

II. MATERIALS AND METHODS

Bacterial strains and inocula preparation

Listeria monocytogenes strains used in this study included five isolates from the International Life Sciences Institute North America (ILSI NA) *L. monocytogenes* strain collection (ILSI no. 7, 11, 29, 35 and 36) (8). The strains were maintained in frozen culture at -80°C until subcultures were prepared by inoculating test tubes with 5 ml of Brain Heart Infusion broth (BHI, Oxoid, England) with a single colony growing in Triptone Soya Agar (TSA, Oxoid, England) supplemented with 0.3% (w/v) yeast extract and 0.1% (w/v) of sodium pyruvate, and incubated at 37°C overnight. The cultures were centrifuged at 7000 rpm at 4°C for 10 min and the pellets washed twice with Ringer solution (Oxoid, England). Then, the suspensions were diluted three times in 9 ml of Ringer solution (Oxoid, England) to give an approximate concentration of 10^6 cfu/ml. The pool was prepared by mixing equal volumes of each strain.

Carpaccio samples preparation and HPP

Bovine *Semitendinosus* muscles were rubbed in by hand into plastic bags (Cryovac HT, Cryovac L.T., Spain) with curing salts (weight of product basis: sodium chloride 1.2%, sodium tripolyphosphate 0.1%, sodium citrate 0.05%, sodium nitrite 0.015%, sodium isoascorbate 0.05%) or with curing salts plus the addition of potassium lactate 3.0% (PURASAL Hi-Pure P Plus, PURAC, Spain) or potassium lactate 1.7% and sodium diacetate 0.12% (PURASAL Opti.Form PD4, PURAC, Spain). Then, muscles were vacuum-packed and stored at 4°C for 12 days. After chill storage, cured muscles were frozen and stored at -20°C until slicing (1.5-2 mm). *Carpaccio* slices (25±1 g) were inoculated with the pool of *L. monocytogenes* strains to obtain a concentration of approximately 10^4 cfu/g. Then, samples were vacuum-packed (Cryovac HT, Cryovac L.T., Spain) and frozen at -60ºC. Frozen *carpaccio* samples were pressurized at 600 MPa for 5 min in a discontinuous hydrostatic pressurization unit (Hiperbaric 135, Hiperbaric, Burgos, Spain). The initial water (pressurization fluid) temperature was 15°C. After treatment, all samples were stored at 4°C and analyzed after 1, 7, 14 and 21 days of storage.

Experimental design and statistical analysis

The experiment was arranged in a complete randomized 3x2 factorial design. The model included the main effects of antimicrobial addition (none, potassium lactate 3.0% or potassium lactate 1.7% and sodium diacetate 0.12%), HPP (0.1 or 600 MPa) and antimicrobial by HPP interactions. Three experimental units were used for each treatment. Then, ANOVA was performed to evaluate significant ($p<0.05$) effect of factors and the Tukey multiple comparisons test ($p<0.05$) was applied to analyze statistical differences among means. Statistical analysis was performed using the SPSS-v12 software (SPSS, Chicago, IL., USA).

Microbiological and chemical analyses

For microbiological analysis, *carpaccio* slices were aseptically removed from their plastic wrapping and stomached (AES laboratoire, France) for 1 min in sterile bags with filter (Bag Page, Interscience, France) with sufficient volume of Ringer solution (Oxoid, England) to obtain an initial 1/10 (w/v) dilution. Successive decimal dilutions were then performed and plated onto appropriate medium. *L. monocytogenes* were determined by plate counting in Chromogenic Listeria Agar (Oxoid, England) supplemented with OCLA (ISO) Selective Supplement (SR0226E, Oxoid, England) and Brillance™ Listeria Differential Supplement (SR0228E, Oxoid, England), after 48 h of incubation time at 37ºC. Lactic acid bacteria (LAB) were determined by plating on Man, Rogosa and Sharpe agar (MRS, Oxoid, England) incubated for 72 h at 30ºC, while psychrotrophs on Plate Count Agar (PCA, Merck, Germany) incubated for 10 days at 8°C.

The water activity (a_w) and pH was determined for each *carpaccio* formulation. Water activity was measured at 25°C using a water activity meter (AquaLab CX-2, Decagon Devices Inc., USA) and pH using a digital pH-meter (Micro pH 2001, Crison, Barcelona, Spain).

III. RESULTS AND DISCUSSION

Water activity (a_w) and pH

In regard to *carpaccio* formulation, the a_w values of samples with curing salts or with curing salts plus potassium lactate or potassium lactate and sodium diacetate were 0.978±0.003, 0.970±0.001 and 0.973±0.003, respectively. In addition, the pH values were 5.47±0.03, 5.80±0.03 and 5.78±0.02, respectively.

Microbiological counts

Table 1 shows the microbial evolution on *carpaccio* samples treated with or without antimicrobials and HPP, during 21 days of storage at 4°C. A significant ($p<0.05$) HPP effect was observed in *L. monocytogenes* counts. It can be seen that HPP was able to reduce the *L. monocytogenes* count below the detection limit (<2 log cfu/g). Moreover, *L. monocytogenes* remained undetectable in all pressurized samples throughout the storage. Regarding unpressurized samples (0.1 MPa), no significant ($p>0.05$) differences were observed in *L. monocytogenes* counts of samples with or without antimicrobial addition, at each day of storage. HPP has been recognized by the Food Safety and Inspection Service of USA as a useful listericidal post-processing and post-packing treatment for RTE foods (9). In addition, several studies reported about the effectiveness of HPP to control *L. monocytogenes* in RTE meat products. In this sense, Jofré *et al.* (10) reported that pressurization of sliced cooked ham at 600 MPa

Table 1. Microbial evolution (log cfu/g) of *carpaccio* samples treated with or without antimicrobials and high pressure, during storage at 4ºC.

	Antimicrobial*	Pressure (MPa)	Days of storage			
			1	7	14	21
Listeria monocytogenes						
	None	0.1	4.31±0.20 ns	4.00±0.30 ns	5.08±0.84 ns	3.70±0.20 ns
		600	<2	<2	<2	<2
	PL	0.1	5.02±0.18 ns	5.38±0.64 ns	4.47±0.47 ns	3.70±0.15 ns
		600	<2	<2	<2	<2
	PL+SD	0.1	4.37±0.51 ns	4.10±0.17 ns	4.37±0.65 ns	3.95±0.30 ns
		600	<2	<2	<2	<2
LAB						
	None	0.1	6.06±0.07 A	7.34±0.17 a	6.67±0.58 a	7.30±0.35 a
		600	2.05±0.95 B	4.81±0.40 b	6.18±0.34 b	7.01±0.41 a
	PL	0.1	5.56±0.08 A	6.52±0.05 a	6.36±0.18 a	6.33±0.06 ab
		600	2.52±0.24 B	3.89±0.04 bc	5.43±0.02 bc	6.31±0.72 ab
	PL+SD	0.1	5.67±0.20 A	6.47±0.11 a	6.39±0.36 a	6.67±0.02 b
		600	<2	3.40±0.45 c	5.29±0.10 c	5.16±0.45 b
Psycrotrophs						
	None	0.1	7.00±0.20 A	8.08±0.09 a	8.43±0.08 ns	nd
		600	4.00±0.18 B	5.84±0.27 c	7.46±1.03 ns	8.28±0.05 ns
	PL	0.1	6.00±0.10 A	7.52±0.08 b	7.30±0.30 ns	nd
		600	3.00±0.24 B	4.41±0.15 d	7.35±0.14 ns	8.08±0.27 ns
	PL+SD	0.1	6.00±0.12 A	7.45±0.10 b	7.77±0.07 ns	nd
		600	3.00±0.30 B	3.86±0.29 e	7.37±0.44 ns	7.82±0.27 ns

*Antimicrobial: none, potassium lactate 3.0% (PL) and potassium lactate 1.7% and sodium diacetate 0.12% (PL+SD).
Means with different capital letters in the same column indicate significant differences ($p<0.05$) regarding pressure effect while means with different lower case letters in the same column indicate significant differences ($p<0.05$) regarding antimicrobial by pressure interaction effect.
ns: non-significant / nd: non-determined.

for 5 min at 10ºC produced a significant reduction of around 3.5 log cfu/g in *L. monocytogenes* counts and samples showed levels lower than 10 cfu/g during 90 days of storage at 1ºC and 6ºC. Also, Koseki *et al.* (11) observed that the number of *L. monocytogenes* on cooked ham inoculated with 10^5 cfu/g was initially reduced by HPP at 500 MPa for 10 min to below the detectable level (10 cfu/g). However, the bacterial count gradually increased during storage at 10ºC, and exceeded the initial inoculum level at the end of the 70-day period.

The analysis of the spoilage microflora of *carpaccio* showed an initial population of LAB and psycrotrophs between 5.5 and 7 log cfu/g depending on *carpaccio* formulation. After HPP (day 1), both microbial counts were significantly ($p<0.05$) reduced by about 3 log cycles. Garriga *et al.* (6) report that marinated beef loin slices treated at 600 MPa for 6 min at 31ºC showed a significant reduction of at least 4 log cycles after treatment for aerobic, psycrothrophic, and LAB counts. Instead, Realini *et al.* (12) informed that frozen cured pork *carpaccio* treated at 600 MPa for 6 min at freezing temperature (−15°C or −35 °C) had only 0.5 and 1.5 log cycle reductions of LAB and psychrotrophs counts, respectively. Regarding antimicrobial addition, significant ($p<0.05$) interaction of HPP and antimicrobial additives was observed in LAB and psycrotrophs counts during storage at 4ºC. Samples with potassium lactate 1.7% and sodium diacetate 0.12% treated at 600 MPa presented a delay in the growth of LAB and the lowest counts (5.16 log cfu/g) at 21 days of storage. This effect was also observed in psycrotrophs count of pressurized samples with potassium lactate 3.0% and with potassium lactate 1.7% and sodium diacetate 0.12% at day 7 of storage. However, psycrothophs counts shown a noticeable increase at day 14 and no significant ($p>0.05$) differences were observed among treatments. In this concern, Diez *et al.* (13) observed that the addition of 3% mixture of potassium/sodium L-lactate to morcilla de Burgos formulation extended the shelf life by

around three weeks respect to control samples but the combination of HPP (600 MPa for 10 min) and lactate salts showed only a small synergic effect on spoilage bacterial population, with no significant difference in comparison to HPP samples.

IV. CONCLUSION

HPP alone was sufficient to ensure a 4 log reduction of *Listeria monocytoges* strains on frozen beef *carpaccio* and increase the shelf life of the product. After HPP, potassium lactate and sodium diacetate combination delayed the growth of LAB and psycrothophs during refrigeration storage. Nevertheless, the shelf life extension achieved in this work has been shorter than expected.

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