Antilisterial efficacy of Lactobacillus *bacteriocins and organic acids on frankfurters. Impact on sensory characteristics*

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ORIGINAL ARTICLE

Antilisterial efficacy of *Lactobacillus* bacteriocins and organic acids on frankfurters. Impact on sensory characteristics

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Abstract Dipping solutions containing bacteriocins produced by *Lactobacillus curvatus* CRL705 and *Lactobacillus sakei* CRL1862 (Bact705/1862), nisin and organic acids (lactic acid, LA; acetic acid, AA) were tested alone or in combination against *Listeria monocytogenes* inoculated by immersion on vacuum-packaged frankfurters stored at 10 °C during 36 days. LA/AA solution (2.5% v/v each) reduced pathogen population by 1.50 $\log_{10}$ CFU/ml during storage. Semi-purified Bact705/1862 prevented *L. monocytogenes* growth, while nisin was not able to avoid its regrowth after 20 days. The combined addition of Bact705/1862 + LA/AA was the most effective approach for pathogen reduction below detection level from day 6 to final storage. Frankfurters treated with Bact705/1862 + LA/AA compared to fresh-purchased samples did not show significant differences in flavor, juiciness, color intensity and overall preference at 22 days-storage at 5 °C. Meat processors should not only validate the antimicrobial efficacy of combined treatments but also their sensory impact on the product, which is directly related to consumer acceptability.

Keywords Biopreservation · Bacteriocins · Frankfurters · Listeria · Organic acids

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Introduction

Listeria monocytogenes is an opportunist food-borne pathogen responsible for listeriosis, a disease associated with high mortality rates. Although a statistically significant growing trend of listeriosis was observed over the period 2008-201, the European Union (EU) notification rate in 2015 was similar to that of 2014 but with the highest annual number of deaths. In 2015, the non-compliance for the different ready-to-eat (RTE) food categories collected at processing was comparable to previous years with smoked fish, dairy products (other than cheeses) and heat-treated meat products being the most implicated (EFSA 2016). In addition, 1600 illnesses and 260 deaths due to listeriosis are reported every year in the United States of America (CDC 2014). The ubiquitous nature of *L. monocytogenes* and its ability to grow at temperatures as low as 2–4 °C makes it a significant threat to the safety of ready-to-eat (RTE) products. This pathogen is capable of adhering and forming biofilms on food-contact surfaces and persisting for long periods (Perez-Ibarreche et al. 2016), the contaminated processing facilities playing an important role in the spoilage of food products. Adhesion properties of *Listeria* may allow persistence and recurrence in plant environments, potentially increasing the chance of product contamination. Furthermore relatively limited control interventions at retail and food service establishments and the lack of a specific regulatory framework, increase the likelihood of introduction of this pathogen into foods.

Muscle RTE-products including red meats, poultry, fish and sea foods have been documented to serve as vehicles for *L. monocytogenes* and listeriosis outbreaks have been associated with the consumption of contaminated RTE foods (Marzocca et al. 2004; Martins and Leal Germano 2011). *L. monocytogenes* are normally eliminated during

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the thermal treatment of RTE meat products, therefore its presence is primarily due to post-thermal mishandling. Since *Listeria* contamination mainly occurs during slicing and packaging after cooking and particularly for frankfurters during the peeling of casings before vacuum-packaging; post-packaging decontamination are critical in RTE meat products (Zhu et al. 2005). The pathogen growth is supported even in packages under vacuum and at refrigeration temperatures, and these products are frequently consumed without reheating and often exposed to temperature abuse explaining why they are ideal vehicle for *Listeria.* Unheated emulsion-style sausages such as frankfurters were classified as a "high risk" product causing listeriosis by the quantitative risk ranking assessment on *L. monocytogenes* in RTE foods (FSIS 2003).

Interventions to lessen the prevalence and levels of *L. monocytogenes* in RTE meat foods involve post-packaging decontamination technologies such as in-package thermal pasteurization, irradiation, high-pressure as well as the use of antimicrobial additives (Zhu et al. 2005). Since many of the chemicals currently licensed for use as food preservatives are increasingly being questioned due to their potential effects on humans, pressure on food suppliers to consider the use of natural alternatives is increasing. Amongst intervention technologies for sanitizing or decontaminating frankfurters before vacuum-packaging, antimicrobial metabolites produced by Lactic Acid Bacteria (LAB) have been considered. These bacteria and their metabolites, traditionally present in fermented foods, are generally recognized as safe and approved as food additives, representing an alternative to the use of chemicals. Lactic and acetic acids can be included in the meat batter, sprayed onto the surface, added as dipping solutions or through active packaging, depending on the type of product to be applied (Ahmed et al. 2015). Lactic acid and their salts have been extensively used in the meat industry to increase flavor and to extent shelf life of RTE products (Byelashov et al. 2008).

LAB and their bacteriocins are of great interest since they may be regarded as natural biopreservatives satisfying the consumer demands for naturally preserved foods. Their use to overcome the post-processing contamination of RTE meat products has been reported; several bacteriocins and their producers (LAB) have demonstrated antilisterial effects in cooked meat products. Nisin, the only commercially approved bacteriocin, has limited applications in meat and meat products, especially if the pH is above 5.0 (Huot et al. 1996). *L. monocytogenes* control in frankfurters has been described using pediocin (ALTA2341) alone or combined with other decontaminating technology (Chen et al. 2004). Moreover, the combination of organic acids solutions and different bacteriocins for the control of *L. monocytogenes* in meat products has been described (Ananou et al. 2010; Koo et al. 2012). In previous works, the production of antibacterial substances by the meat isolates *Lactobacillus curvatus* CRL705 and *Lactobacillus sakei* CRL1862 was reported (Vignolo et al. 1993; Castro et al. 2011; Hebert et al. 2012). While the genome analysis of *L. curvatus* CRL705 revealed the presence of genes coding for five bacteriocins production (Hebert et al. 2012), only lactocin 705 (a two-peptide bacteriocin) and lactocin AL705 (an antilisteria bacteriocin) have been characterized; the latter being highly inhibitory when applied in meat systems and vacuum-packaged meat (Castellano et al. 2008). On the other hand, *L. sakei* CRL1862 has demonstrated to produce antimicrobial compound/s able to inhibit *L. monocytogenes* (Castellano et al. 2012). Although the use of organic acids and bacteriocins has been documented to be effective in controlling the growth *L. monocytogenes*, they have the potential to negatively affect product quality and consumer acceptance. Therefore, the aim of this study was to investigate the effectiveness of antilisteria bacteriocins produced by meat borne bacteriocinogenic lactobacilli, nisin and organic acids alone or in combination as aqueous dipping solutions to reduce surface-inoculated *L. monocytogenes* on commercially manufactured vacuum-packaged frankfurters under temperature abuse. Furthermore, the impact on frankfurters sensory quality was investigated after the application of acid mixtures and its combination with semi-purified bacteriocins.

Materials and methods

Bacterial strains, culture conditions and inoculum preparation

Lactobacillus curvatus CRL705 and *Lactobacillus sakei* CRL1862 from CERELA culture collection were previously isolated from artisanal dry sausages (Vignolo et al. 1993; Castro et al. 2011) and routinely grown in MRS broth at 30 °C for 18 h. *Listeria monocytogenes* FBUNT (Facultad de Bioquímica, Química y Farmacia, UNT, Argentina) and *L. monocytogenes* SR215 (Institute of Hygiene and Toxicology, IHT, Karlsruhe, Germany) were grown in TSBYE (Tryptic Soy Broth, supplemented with 0.5% yeast extract, pH 6.7) at 30 °C for 24 h. Unless otherwise stated, the used microbiological media were supplied by Britania (Argentina). For inoculum preparation, *L. monocytogenes* FBUNT and SR215 were separately grown in 10 ml of TSBYE at 30 °C for 18 h to give $\sim$ 9.0 $\log_{10}$ CFU/ml. Cells were harvested by centrifugation (9000×*g*, 10 min at 4 °C), the supernatant discarded and pellets resuspended in sterile 0.85% saline after washing twice by vortexing in the same solution. An

adequate volume of each *L. monocytogenes* culture was added to 1 L of 0.85% saline solution to provide a 2-strain mixture ($\sim 6.0_{10}$ log CFU/ml) to produce a final cell count of $\sim$ 3.0–4.0 $\log_{10}$ CFU/ml on frankfurters immediately after inoculation.

Partial purification of bacteriocins

One liter from an overnight culture of *L. curvatus* CRL705 or *L. sakei* CRL1862 in MRS broth was centrifuged (11,000×*g*, 20 min at 4 °C), (Avanti J-25I centrifuge, Beckman, USA) to remove bacterial cells and, bacteriocins were precipitated from supernatants with 60% saturated ammonium sulphate (Cicarelli-Reagents S.A). The mixture was held at 4 °C during 30 min with stirring and then centrifuged (11,000×*g*, 10 min at 4 °C). The hydrophobic superficial pellicle was collected by filtration (Whatman paper, Argentina) and solubilized with the bottom pellet in 30 ml of 20 mM phosphate buffer (pH 6.5). This solution was loaded onto a Sep-Pak C18 cartridge (500 mg, Mega Bond Elut, Strata, Agilent, USA) previously equilibrated with 40 ml of MQ water. The cartridge was washed with 12 ml of 0.1% acetic trifluoracetic acid and the adsorbed bacteriocin was eluted with 12 ml of 40% (v/v) acetonitrile in MQ water. Finally, the trifluoracetic acid was evaporated by drying under reduced pressure with a Speed-Vac concentrator (Thermo Savant Instruments, NY, USA). The semi-purified bacteriocins solution obtained presented a brownish color; its antimicrobial activity was determined by a well diffusion assay according to Castellano et al. (2008). A commercial preparation of nisin (2.5% nisin in denatured milk solids and salt, AMG, Bs. As., Argentina) was also used and the stock solution was prepared by dissolving 0.1 g of nisin in 10 ml of 0.02 N HCl and sterilized by filtration through 0.22 mm filters (Millipore, Bedford, MA) before stored at − 4 °C. For experiments, the stock solution was diluted with sterilized 0.02 N HCl to obtain a nisin working solution of 2.500 UI/ml. Lactic and acetic acids (Cicarelli, Bs. As., Argentina) were used at a concentration of 2.5% (v/v).

Antimicrobial treatments of frankfurters

All-beef frankfurters produced by a major meat processor from Argentina were obtained from a local supermarket. Treatments were performed by dipping frankfurters (108 units) into 1 l of solution containing both *Listeria* strains during 2 min and drained for approximately 5 min at 10 ± 2 °C. Then, inoculated frankfurters were exposed to different treatments during 2 min as follows: (T1) saline solution (control); (T2) semi-purified bacteriocins produced by *L. curvatus* CRL705 (533 UA/ml) + *L. sakei* CRL1862 (266 UA/ml); (T3) nisin 2.500 UI/ml; (T4) 2.5% lactic acid (LA) + 2.5% acetic acid (AA); (T5) LA and AA + semi-purified bacteriocins, and (T6) LA and AA + semi-purified bacteriocins + nisin. The pH values of the prepared solutions were 2.50 ± 0.06 (lactic acid + acetic acid), 6.44 ± 0.08 (bacteriocins produced by *L. curvatus* CRL705 and *L. sakei* CRL1862) and 1.89 ± 0.06 (nisin). After draining, frankfurters from each treatment were placed in a vacuum bag (oxygen transmission rate 10–30 $cm^3/m^2/atm^1/24$ h at 25 °C and 75% RH, Cryovac, Argentina), vacuum-packaged (Turbovac 320 ST vacuum packaging machine, Howden Food Equipment, Holland) and stored for 36 days at 10 °C. This condition was chosen to simulate a potential temperature abuse condition during distribution, retail and home storage.

Frankfurters sampling

Duplicate from each treatment were sampled at 0, 3, 6, 8, 14, 17, 22, 28 and 36 days of storage. For the microbiological determinations, 25 g was taken aseptically and mixed (1:10) with sterile saline solution (NaCl 0.85%). Sample homogenization was done in a Stomacher Lab-blender (model 400, Seward Laboratory, London, UK) for 2 min, then serial 10-fold dilutions and plating on the following media was carried out. Plate Count Agar (Britania, Argentina) for total viable counts (TVC), MacConkey agar (Britania, Argentina) for coliforms and PALCAM agar base added with PALCAM *Listeria* Selective Supplement (Difco Laboratories, Inc., Detroit, MI, USA) for *L. monocytogenes* were used. Plates were incubated at 30 °C for 48 h with the exception for MacConkey plates that were incubated at 48 h at 37 °C. For pH measurement, a Metrohn 692 pH/ion meter was applied in frankfurters homogenates.

Antimicrobial activity determination

Portions (3 cm^2) from the side surface of the frankfurters subjected to each treatment were cut and placed in semi-solid TSBYE plates overlay inoculated with *L. monocytogenes* FBUNT. Positive bacteriocin activity was evidenced as an inhibition zone on the indicator organism lawn around the frankfurters portions.

Sensory analysis

Sensory analysis was performed to evaluate consumer preference of uninoculated frankfurters treated with LA (2.5%) + AA (2.5%) solution (T4) and acids solution + semi-purified bacteriocins (T5). First, differences among fresh-purchased samples treated with acids

solution and acids solution + semi-purified bacteriocins were evaluated, while in a second session, the effect of 22-days storage at 5±1 °C of treated vacuum-packaged frankfurters was assessed and compared with fresh-purchased treated samples. This temperature, as normal for refrigerators, was selected to evaluate treatment effects on sensory attributes of frankfurters. For the first step, samples were prepared by immersion (2 min) of frankfurters in each solution (T4 and T5). After drainage (5 min) of the samples on a perforate tray, they were refrigerated (5 ± 1 °C) until served to the panel. Half of the samples from each treatment were used the same day for the first trial and the rest were vacuum-packaged and stored under refrigeration during 22 days to perform the second phase of the test. An untrained 28-members panel who regularly consumed this kind of products was recruited to evaluate frankfurters for juiciness, flavor intensity, color and overall preference. Prior to evaluation, an introductory session was held to familiarize panelists with the product. To minimize any residual effect, panelists were instructed to rinse their mouth with water, to eat unsalted crackers between samples and to take sufficient time to score them. Frankfurters were re-heated in boiling water for 3 min, drained and after discarding the extremes, pieces (4 cm) were immediately cut to keep warm and served to evaluators in capped odorless and thermal plastic dishes. Panelists were asked to categorize their liking for each attribute (from less to very much liking). For the first session, a simple paired test was conducted (IRAM 1997); the numbers of consensual answers were computed and a sign test with an α-error < 0.05 was applied. The order that each evaluator assigned to frankfurters (from low to high intensity of color) was recorded. For the second assay, the non-parametric statistical Friedman test for each attribute (juiciness, flavor intensity, overall preference and color) was used. For color evaluation, samples were supplied to the panelists on black dishes in a color cabinet using the CIE standard illuminant D65 (representing average daylight).

Statistical analysis

The experiment was repeated two times. For each experiment, frankfurters were randomly distributed in treatments according to a factorial design 6 × 9 (6 treatments and 9 days). Two experimental units were used in each treatment and time combinations. Data were analyzed using the method of analysis of variance taking a general linear model. In order to analyze the results from sensory analysis a Friedman Test was carried out and minimum significant differences were calculated.

Results and discussion

Antimicrobial effect of organic acids and bacteriocins

In this study, the 2-strain composite of *L. monocytogenes* were able to grow on untreated frankfurters at 10 °C (considered as a temperature abuse condition), increasing their numbers from an initial value of 3.40–5.24 $\log_{10}$ CFU/ml after 36 days of storage (Fig. 1a). The acids mixture (T4) applied after inoculation reduced pathogen population by 1.50 $\log_{10}$ CFU/ml at the end of storage

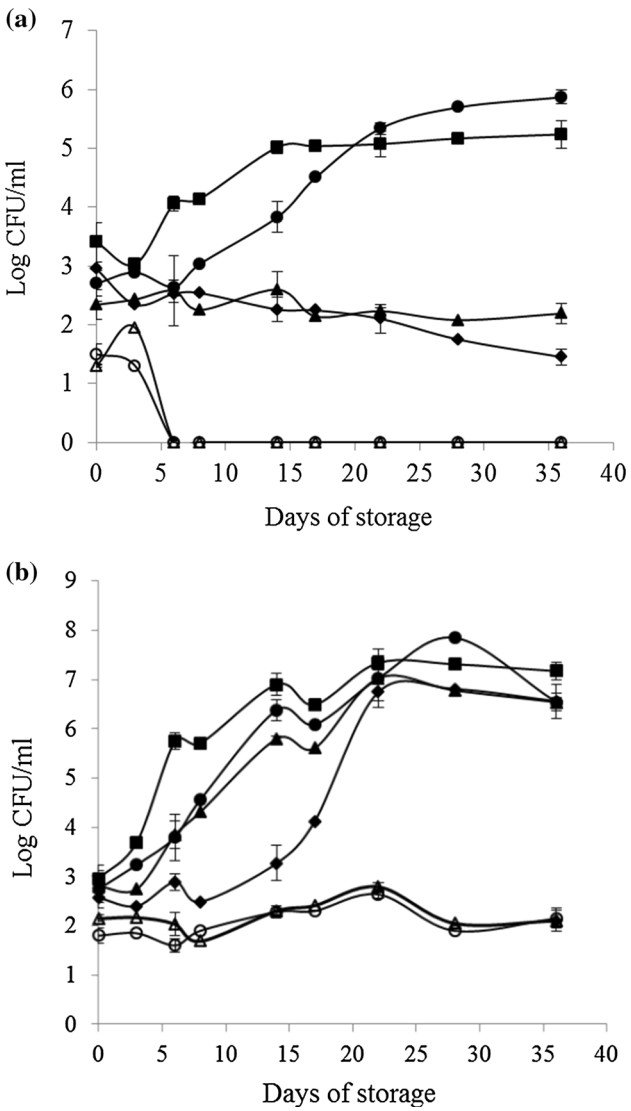

Fig. 1 Growth of *L. monocytogenes* (**a**) and psychrophilic microbiota (**b**) on the surface of frankfurters with and without antimicrobials during 36 days at 10 °C. (Solid square) T1, control; (solid triangle) T2, lactobacilli bacteriocins (*L. curvatus* CRL705 and *L. sakei* CRL1862); (solid circle) T3, nisin; (solid diamond) T4, organic acids; (open triangle) T5, lactobacilli bacteriocins + organic acids; (open circle) T6, lactobacilli bacteriocins + organic acids + nisin

time. Similarly, effective *L. monocytogenes* control by using organic acids solutions as post-processing dipping treatment of inoculated frankfurters after storage at refrigeration temperatures ($\leq$ 10 °C) was reported (Barmpalia et al. 2004). In addition, inhibition of *L. monocytogenes* throughout the shelf life by mixing acids solutions in the formulation of frankfurters was provided during storage at 4 °C (Morey et al. 2014). Moreover, spraying frankfurters with a mixture of lactic acid and sodium lauryl sulphate was also described as a useful antilisterial alternative treatment for RTE meat and poultry products (Byelashov et al. 2008). Thus, utilization of GRAS antimicrobial agents such as various organic acids and salts, in the right concentrations and/or combinations may contribute to the safety of RTE meat foods by preventing proliferation of *L. monocytogenes* during storage. On the other hand, when frankfurters were immersed in the solution containing semi-purified bacteriocins (T2), *L. monocytogenes* growth was completely prevented (listeriostatic effect) whereas nisin addition (T3) resulted in pathogen population decrease during 5 days of storage at 10 °C, then a pathogen re-growth occurred with numbers exceeding that of the control (Fig. 1a). A lower efficiency of the bacteriocins produced by lactobacilli (CRL705 and CRL1862) comparing to acids solutions was obtained. This result may be explained by the interaction of bacteriocins with food components as well as other additives. Particularly for emulsions as frankfurters, functionality of antimicrobial peptides will depend on the interactions that peptides may exert within the different emulsion phases, the aqueous phase being considered a key factor because this is where microbial growth takes place (Campos et al. 2013). Although bacteriocin inactivation would be less pronounced in heat-treated meats due to the loss of free sulfhydryl groups during cooking as a result of glutathione-proteins reaction (Stergiou et al. 2006), adsorption to muscle proteins, degradation by proteolytic enzymes and the presence of high fat content in emulsion-like foods may contribute to antimicrobial activity decrease (Katla et al. 2002). Therefore, a comprehensive approach to the functionality of a bacteriocin in an emulsion should consider not only the antimicrobial effectiveness *per se* but also every component of the food matrix. Studies with sakacin P and nisin applied to different muscle products involved several limitations derived from their interaction with phospholipids emulsifiers and other food constituents (Campos et al. 2013). Fat was also shown to be responsible for a large loss of bacteriocins activity when the functionality of *L. sakei* CTC494 was analyzed in a simulated sausage fermentation conditions (Leroy and De Vuyst 2005). Particularly, the lack of *L. monocytogenes* inhibition by nisin in this study could be explained to the poor solubility at pH $\geq$ 6.0, the effect of low temperature on membrane fluidity, the interference with meat phospholipids and inactivation by formation of a nisin-glutathione adduct as previously reported (Stergiou et al. 2006). In addition, sensitivity variations of *L. monocytogenes* FBUNT and SR215 here used to different bacteriocins were previously determined and an enhanced tolerance to nisin when compared to non-nisin bacteriocins was observed (Castellano et al. 2001). Nisin resistance response of *L. monocytogenes* strains may be correlated with alterations within cell envelope and fatty acids composition that may prevent pore formation (Kramer et al. 2006).

When the combination of antimicrobials in the dipping solution was assayed, a significant greater inhibition of *L. monocytogenes* growth was produced (Fig. 1a). The addition of lactobacilli semi-purified bacteriocins + organic acids to the dipping solution (T5) resulted in an initially *Listeria* growth up to 2.00 $\log_{10}$ CFU/ml at day 3, but then a pronounced decrease to non-detectable levels at day 6 occurred, this effect being maintained up to the end of storage period. Furthermore, when the combination of semi-purified bacteriocins + organic acids + nisin (T6) was applied on frankfurters, a more dramatic decrease to non-detectable cells was produced. Non-viable *Listeria* cells from day 6 to the end of storage was found, a listericidal effect being observed on frankfurters during storage. In agreement with these results, the combination of enterocin AS-48 with chemical preservatives, highly improved the antilisteria effect in non-fermented meat foods such as cooked ham stored at 5 °C (Ananou et al. 2010). In frankfurters containing lactate/diacetate (2.1/0.15%), the addition of Lactiguard® (three antilisteria LAB strains) plus their cell free extracts resulted more effective in reducing growth of *L. monocytogenes* after 8 weeks of refrigerated storage (Koo et al. 2012). In addition, it has been reported that combination of LAB bacteriocins would have a greater effect against *Listeria* than when used individually. In fact, the use of a combination containing bacteriocins belonging to different classes proved to be more effective in preventing regrowth of *Listeria* (Kaur et al. 2013). As bacteriocins used in this study belong to different classes (nisin, class I; lactocin 705, class IIb; lactocin AL705 and the antilisteria compound/s produced by *L. sakei* CRL1862, class IIa), it may be assumed that this mixture would be bactericidal to more cells in a sensitive population, since cells resistant to one bacteriocin would be killed by another. In addition, it is well known the resistance of *L monocytogenes* cells to nisin (Collins et al. 2010). Although initial differences in *Listeria* growth were observed before day 6 (Fig. 1a, T5 and T6), similar *L. monocytogenes* inhibition patterns were then obtained, a non-significant contribution of nisin could be assumed. Organic acids and their salts greatly potentiate the activity of bacteriocins, while acidification enhances the

antibacterial activity of both organic acids and bacteriocins; the increase in the net charge of bacteriocins at low pH would facilitate translocation of bacteriocin molecules through the cell wall (Gálvez et al. 2007). Lacticin 3147 activity also increased in combination with sodium lactate or sodium citrate (Scannell et al. 2000), the same effect being produced for pediocin AcH activity in combination with sodium diacetate and sodium lactate (Uhart et al. 2004).

When the psychrophilic microbiota naturally present in frankfurters was evaluated (Fig. 1b), an increase in cell numbers from 2.96 to 7.17 $\log_{10}$ CFU/ml at the end of storage was found. Similar to that observed for *L. monocytogenes*, a growth prevention of psychrophilic population only occurred when combined treatments (T5 and T6) were applied on frankfurters. The addition of lactobacilli bacteriocins (T2) or nisin (T3) alone were unable to reduce natural bacterial population growing at 10 °C, counts at 36 days being similar to that of the control. However, a bacteriostatic effect was observed up to 8 days when 2.5% LA/AA (T4) were added to the dipping solution, and then an increase of the psychrophilic microbiota by 4 $\log_{10}$ CFU/ml from days 8 to 22 occurred. When the presence of coliforms during 36 days was evaluated, none of the samples contained detectable levels (data not shown). As stated above, the presence of organic acids or their salts significantly potentiate the antimicrobial activity of nisin and non-nisin bacteriocins; the solubility of bacteriocins may also increase at lower pH, facilitating their diffusion (Gálvez et al. 2007).

Stability of bacteriocins during frankfurters storage

The presence of inhibitory activity in frankfurters during the 36 days of storage at 10 °C was determined (Table 1). Frankfurters surface from the control series (T1) did not exhibit any inhibitory activity during the complete experiment, while antibacterial activity was detected when lactobacilli bacteriocins alone (T2) or combined with organic acids (T5) were present in the dipping solution. However, bacteriocins produced by *L. curvatus* CRL705 and *L. sakei* CRL1862 were able to exert antilisteria effect on frankfurters during 36 days of storage; the presence of bacteriocins on the product was revealed by the inhibition of *L. monocytogenes* growth. In spite of this, antilisteria activity in samples treated with nisin (T3) was only detected up to 14 days of incubation, these results being in correlation with the observed *L. monocytogenes* growth (Fig. 1a). This is in agreement with previous results that reported the biopreservative culture *L. curvatus* CRL705, producer of the bacteriocins lactocin 705 and lactocin AL705, exhibited antimicrobial activity on vacuum-packaged meat discs from 0 to 36 days of incubation at 2 °C (Castellano et al. 2008). Likewise, the presence of antilisteria activity in a beaker sausage inoculated with *L. sakei* CRL1862 from the second day until the end of incubation at 22 °C was also detected (Castellano et al. 2012). Also, sakacin P and AS-48 bacteriocins recovery was reported during storage of chicken cold cuts and sausages (Katla et al. 2002; Ananou et al. 2005).

Table 1 Antimicrobial activity on frankfurters subjected to temperature abuse conditions (10 °C during 36 days)

Treatment/day	Antimicrobial activity[a]								
	0	3	6	8	14	17	22	28	36
1	−	−	−	−	−	−	−	−	−
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	−	−	−	−
4	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+

[a]Against *Listeria monocytogenes* FBUNT; − no inhibition, + inhibition zone around the frankfurters portions > 3 mm

Physicochemical analysis

Changes in pH of frankfurters subjected to temperature abuse (10 °C) for the different treatments are shown in Table 2. During storage, a slight decrease in pH values in the presence of organic acids either alone or in combination with the bacteriocins, was found. The pH of control samples (day 0) was 6.68 ± 0.02 and reductions of 0.67, 0.65 and 0.58 pH units for treatments with LA/AA (T4), lactobacilli bacteriocins + LA/AA (T5) and lactobacilli bacteriocins + nisin + LA/AA (T6), respectively were obtained. Dipped frankfurters in (2.5%) LA were found to reduce the pH by 0.3–0.4 pH units while in the presence of (2.5%) AA pH reduction was 0.2–0.4 during the first 10 days of storage at 10 °C (Barmpalia et al. 2004). However, no effect on pH values compared to control was observed when pediocin AcH preparation was added to slice cooked sausages (Mattila et al. 2003).

Sensory analysis

The feasibility of using organic acids (LA, 2.5% + AA, 2.5%) solution and organic acids + semi-purified bacteriocins combination (treatments T4 and T5, respectively) as dipping solutions for superficial antilisterial treatment of frankfurters was examined by a sensory analysis. To be practically effective, such treatments on frankfurters should have no adverse effects on product quality attributes, therefore evaluations by untrained sensory panel were performed to replicate consumer preference of treatments.

Table 2 pH values of frankfurters subjected to temperature abuse conditions (10 °C during 36 days)

Treatment/day	pH						
	0	3	8	14	22	28	36
1	6.68 ± 0.02^{d*}	6.56 ± 0.06^{b}	6.50 ± 0.16^{b}	6.48 ± 0.01^{c}	6.52 ± 0.05^{d}	6.47 ± 0.01^{c}	6.52 ± 0.04^{b}
2	6.44 ± 0.02^{c}	6.42 ± 0.12^{b}	6.38 ± 0.02^{b}	6.45 ± 0.03^{c}	6.42 ± 0.02^{c}	6.49 ± 0.03^{c}	6.50 ± 0.01^{b}
3	6.37 ± 0.04^{c}	6.43 ± 0.04^{b}	6.42 ± 0.01^{b}	6.44 ± 0.01^{c}	6.40 ± 0.02^{c}	6.46 ± 0.01^{c}	6.51 ± 0.02^{b}
4	6.01 ± 0.05^{a}	6.09 ± 0.11^{a}	6.10 ± 0.08^{a}	6.13 ± 0.01^{ab}	6.01 ± 0.01^{a}	6.17 ± 0.03^{a}	6.22 ± 0.05^{a}
5	6.03 ± 0.03^{ab}	6.05 ± 0.07^{a}	6.06 ± 0.03^{a}	6.19 ± 0.02^{b}	6.16 ± 0.02^{b}	6.24 ± 0.02^{b}	6.21 ± 0.05^{a}
6	6.10 ± 0.03^{b}	6.13 ± 0.01^{a}	6.05 ± 0.02^{a}	6.08 ± 0.06^{a}	6.13 ± 0.02^{b}	6.26 ± 0.03^{b}	6.26 ± 0.05^{a}

*Mean ± standard deviation; means with the same letters (a, b, c, d) are not significantly different ($p < 0.05$)

Table 3 Sensory characteristics of uninoculated and treated frankfurters with acids mix (T4) and acids mix + semipurified bacteriocins (T5)

Treatments	Juiciness		Flavor intensity		Overall preference		Color intensity	
	0^{a}	22	0	22	0	22	0	22
T4	81^{a}*	57c	63a	67a	72a	63a	80a	26b
T5	63ab	59bc	63a	67a	57a	68a	78a	76a

Scores varied from 28 (panelists number) to 112 (28 × 4 attributes)

*Means with same letters (a, b, c) are not significantly different for the same attribute

[a]Sampling time (0 and 22 days)

Sensory analysis of uninoculated frankfurters was conducted initially (fresh-purchased frankfurters) and after 22 days of vacuum-packaging storage at 5±1 °C. Although frankfurters analysis was separated in two sessions, no effect of session was found by variance analysis (data not shown). Firstly, paired test (fresh-purchased treated samples) showed frankfurters did not differ significantly for juiciness and preference, however samples from T5 exhibited a higher flavor intensity (significance level, α: 10%) and a darker color (significance level, α: 5%) while no odd flavors were detected (data not shown). On the other hand, when Friedman test was applied (Table 3), samples from T4 and T5 after 22 days of refrigerated and vacuum-packaged storage, showed less juiciness (57 and 59 for T4 and T5, respectively) compared to fresh-purchased (81 and 63 for T4 and T5, respectively). Fresh-purchased frankfurter samples treated with organic acids (T4) were found as the juiciest. These observations disagree with Morey et al. (2014) who reported that beef frankfurters treated with sodium lactate (2%) and sodium diacetate (0.25%) solution and stored at 4 °C were significantly less preferred across all sensory attributes, mainly due to their dry, rubbery, and acetic acid odor. Similarly, the use of organic acids as marinades had a negative effect on quality attributes of cooked meat quality of turkey deli loaves (Carroll et al. 2007). On the other hand, no significant differences were obtained for flavor intensity and overall preference for samples treated with organic acids (T4) stored during 22 days (Table 3). Similarly, when sodium lactate/sodium diacetate were included in frankfurters formulation and dipping solution, flavor and overall acceptability were not affected compared to controls (Barmpalia et al. 2004). In addition, sensory characteristics of beef strip loins were found unaffected after 112 days of storage after treatment of LA and AA (2%) as reported by Mikel et al. (1996). When color changes were analyzed, a lower score for frankfurters treated with LA/AA solution (T4) was assigned by panelists (Table 3). In agreement with this study, significant decrease in the redness and increase in lightness values were reported for frankfurters treated with organic acid salt solutions (Lu et al. 2005). Nevertheless, the superficial discoloration observed for frankfurters in T4 samples (lower score) could be attributed to the effect of organic acids on meat protein denaturation. However, higher color score for T5 samples could be explained by the brownish color of semi-purified bacteriocins included in antimicrobial solution that could have been masked organic acids effect. Although first exploratory analysis showed darker color and higher flavor intensity for fresh-purchased frankfurters after treatment with antimicrobials combination, these attributes for T5 sample were no significantly different after 22 days of storage. These results suggest that the presence of semi-purified bacteriocins in the dipping solution would not greatly affect frankfurters sensory attributes.

Conclusion

Dipping solution containing semi-purified bacteriocins + acids mix (T5) proved to be the most effective treatment in suppressing the growth of *L. monocytogenes* on frankfurters during refrigerated and vacuum-packaged storage. Sensory analysis of uninoculated and treated frankfurters showed no impact of the used antimicrobials combination on overall product acceptability. In view of extending shelf life and inhibiting *L. monocytogenes* in RTE meat products, meat processors should consider the addition of natural antimicrobial compounds such as bacteriocins and organic acids, evaluate their efficacy and also test if these affect consumer acceptability.

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