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Combined effect of pediocin bacHA-6111-2 and high hydrostatic pressure to control *Listeria innocua* in fermented meat sausage

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Abstract

In and ex situ production of bacteriocin (bacHA-6111-2) combined with high hydrostatic pressure (300 MPa, 5 min, 10ºC) were assessed on the survival of *Listeria innocua* previously inoculated in Alheira, a traditional Portuguese fermented meat product. The effect was evaluated immediately after each treatment, alone and combined, and during 60 days of storage (4ºC). For higher concentrations of *L. innocua,* a bacteriostatic effect was verified: i) during the first days of storage for the pressure treatment alone and in combination with ex situ production of bacteriocin; and ii) for longer periods, when pressure was combined with in situ production of bacteriocin. After this effect, the first treatments revealed rapid growth up to the initial values; while the latest treatment, and for lower initial cells, resulted in a decrease > 2 log CFU g^{-1}, from day 3 of storage until the end of the study. A similar result was obtained for analogous pressurized *L. innocua* samples with ex situ production of bacteriocin. The developed work has demonstrated the potential of using the combination of natural antimicrobial compounds (pediocin bacHA-6111-2, produced either in or ex situ) with mild pressure (300 MPa, 5 min) treatments to control effectively *L. innocua* in fermented meat products such as Alheiras, when contaminated with values closed to the ones that might potentially arise by natural post-contamination.

Introduction

Health-conscious consumers are continuously looking for "natural" and "traditional" foods, preferably without chemical preservatives. Starters and protective cultures such as lactic acid bacteria (LAB) have been used in fermented foods in order to guarantee the homogeneity, quality and safety of the products (Holzapfel and Wood, 2014).

Alheira is a traditional and naturally fermented meat sausage, typical of the North of Portugal. For the production of Alheira, the various meats are boiled in water with salt and spices. Bread is thinly sliced and immersed in some of the broth formed during the boiling of the meats and when it is soft enough, meat in small pieces, spices and olive oil and/or fat drippings are added to the mixture. When everything is completely mixed the paste is studied into cattle intestinal or cellulose-based casings and submitted to a dry smoke process, usually for no longer than eight days. A wide variety of microorganisms have already been isolated from Alheira, including foodborne pathogens e.g. *Listeria monocytogenes, Salmonella* spp., *Staphylococcus aureus* and *Clostridium perfringens* (Ferreira *et al.*, 2007) and autochthonous LAB cultures e.g. *Pediococcus acidilactici* and *Lactobacillus plantarum* that have antimicrobial effect (Albano *et al*., 2007; Todorov *et al.*, 2010).

High pressure processing (HPP) is actually considered one of the most promising alternative food processing techniques for microbial inactivation without affecting the organoleptic properties and nutritional value, as well as having no harmful impact on the environment. HPP treatment applied to meat and related meat products as already been reported (Simonin *et al*., 2012); the combination of technologies, is also considered a tool to achieve food safety (Marcos *et al.*, 2005; de Alba *et al*., 2013). Application of HPP will impact negatively the products price, especially if processed at pressures greater than 600 MPa limiting the application of this technology.

The application of bacteriocins in food preservation can offer many benefits namely the

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possibility to use less severe treatments without compromising the food safety aspects, achieving better preservation of food nutrients and vitamins, as well as sensorial properties of food (Davidson, 2006; Abdel Ghany, 2015). Bacteriocins are generally well accepted, recognized as safe and effective and some are commercial available (e.g. nisin) (Abdel Ghany, 2015). However, disadvantages like inactivation in food matrices or emergence of resistant mutants might occur. To avoid these limitations, the combined use of bacteriocins with other processes i.e. HPP should be taken in consideration (Simonin *et al*., 2012; de Alba *et al*., 2013). Presently, according to the European legislation, addition of bacteriocins as food preservatives requires specific legislation (Directive 95/2/EC). An industrial challenge might be the in situ application of bacteriocins by the addition of the bacteriocinogenic LAB to the food matrix. Recently, de Alba *et al.* (2013) reported that in situ and ex situ production of bacteriocins in combination with HPP seemed to be a feasible procedure to improve safety of meat products. However, process parameters should be established for every food matrix prior to industrial use for food safety and shelf life extension.

The aim of the present study was to assess the combined effect of pediocin bacHA-6111-2 and mild hydrostatic pressure (300 MPa, 10ºC, 5 min) to control *Listeria innocua*, immediately after the treatments and during refrigerated storage, in a traditional meat sausage (Alheira). This work assembles the combination of pressure, under the commercial range (400-600 MPa), with both in and ex situ production of a pediocin in a real food matrix, to control *L. innocua* in fermented meat under refrigerated storage. This research will allow eliminating speculations towards comparisons that are done between the effects of similar combined treatments presented in separate papers, which often use different experimental methodologies.

Materials and Methods

Bacterial strains

Listeria innocua 2030c (Culture Collection, Escola Superior de Biotecnologia, Porto, Portugal) was grown on Tryptone Soya agar with Yeast Extract 0.6% (w/v) (TSAYE, Lab M, Bury, United Kingdom) and subcultured in Tryptone Soya broth with Yeast Extract (TSBYE, Lab M) at 37°C for 24 h. Two different pediococci strains were used: *P. acidilactici* HA-6111-2, a strain with antilisterial activity (Albano *et al*., 2007), and *P. acidilactici* (HA-2485-3), a non bacteriocinogenic culture (negative control for bacteriocin production). Both *P. acidilactici* cultures were cultured on de Man, Rogosa and Sharpe (MRS) agar (Lab M) and subcultured twice in MRS broth (Lab M), at 37°C, for 18 to 24 h. Cells were harvested under aseptic conditions by centrifugation (6000 x g, 10 min, 4ºC). Cell pellets were washed twice in sterile Ringer's solution (Lab M) and resuspended in i) TSBYE and MRS broth for *L. innocua* and *P. acidilactici*, respectively for bacteriocin assays and ii) in Ringer's sterile solution for Alheira inoculation until reach a concentration of *L. innocua* (ca. 105, 107 and 109 CFU mL^{-1}) and both strains of *P. acidilactici* (ca. 109 CFU mL^{-1}).

Crude bacteriocin extract

Pediococcus acidilactici HA-6111-2 culture supernatant obtained as described above, was adjusted to pH 6 by the addition of NaOH (1 mol L^{-1}) and heated at 85ºC (10 min). Bacteriocin (bacHA-6111-2) activity was measured against *L. innocua* and expressed as arbitrary units (AU) per mL (van Reenen *et al*., 1998). The initial bacteriocin activity in the supernatant was 6400 AU mL^{-1}.

Preparation of Alheiras

Non-smoked paste of Alheira was directly obtained from a batch production in an industrial meat company located in the North of Portugal (Minhofumeiro, Ponte de Lima Portugal). Samples were collected and transported to the lab in refrigerated conditions. Before lab assays, paste of Alheira was sterilized at 121ºC (15 min). One hundred grams of paste was inoculated with 5 mL of bacteria(s) and/or bacteriocin; each sample was then gently mixed manually in sterile stomacher bags (ca. 3 min). Small Alheira sausages (ca. 5 g) were aseptically prepared and stuffed into casings (cellulose φ 28 mm, kindly supplied by Primor, Portugal) and thereafter stored overnight at 4ºC, for a period of 18 hours. The experimental conditions related to the preparation of the Alheira pastes were: (1) sterile paste (un-inoculated); (2) inoculated with *L. innocua,* at different cell numbers (4, 6 and 8 log CFU g^{-1}); (3) inoculated with *P. acidilactici* (HA-6111-2); (4) inoculated with *L. innocua*, at different cell numbers (4, 6 and 8 log CFU g^{-1}), and *P. acidilactici* (HA-6111-2); (5) inoculated with *P. acidilactici* (HA-2485-3); (6) inoculated with *L. innocua*, at different cell numbers (4, 6 and 8 log CFU g^{-1}), and *P. acidilactici* (HA-2485-3); (7) with pediocin bacHA-6111-2 (320 AU g^{-1}); and (8) pediocin bacHA-6111-2 (320 AU g^{-1}), and *L. innocua*, at different cell numbers (4, 6 and 8 log CFU g^{-1}). Both LAB strains were inoculated at ca. 8 log CFU g^{-1}. For each of the above mentioned condition, the pastes were further divided into two

portions: one was maintained as a control (untreated), while the other was pressure processed.

High hydrostatic pressure treatment

The pressure treatment was carried out using a hydrostatic press from Unipress Equipment, Model U33 (Warsaw, Poland), with a vessel of 100 mL (35 mm diameter, 100 mm height), surrounded by an external jacket, connected to a thermostatic bath to control the temperature. The maximum working pressure can be 700 MPa, within a temperature range of -20ºC and 100ºC. The pressure-transmitting fluid was a mixture of propylene glycol and water (60:40).

For the inactivation studies of LAB strains (*P. acidilactici* HA-6111-2 and HA-2485-3) in Alheiras, a range of pressures between 300 and 600 MPa (10ºC, 5 and 15 min) was performed in duplicate. The combined effect of pediocin bacHA-6111-2 (in and ex situ production) and 300 MPa (10ºC, 5 min) in fermented meat sausage to control *L. innocua* was then assessed under refrigerated storage (4ºC). Before pressurization, small Alheiras were aseptically double packed in low permeability polyamide-polyethylene bags (PA / PE-90, Albipack-Packaging Solutions, Águeda, Portugal), and vacuum-sealed.The compression and decompression rates were, respectively, 43 MPa s^{-1} and 100 MPa s^{-1}. The pressurization period reported in this study did not include the pressure increase or decrease. After depressurization, the samples were immediately cooled in an ice-water bath. Each treatment was carried out in duplicate.

Microbiological analysis

Cells were enumerated before stuffing the small Alheiras, immediately before and just after the pressure treatments (time 0), and during the refrigerated storage (1, 3, 7, 14, 21, 28, 44, and 60 days). At the same time, the controls (non-pressurized) pastes were also analysed. Briefly, each small Alheira from each independent high-pressure treatment was aseptically added to 45 mL of sterile Ringer's solution and further homogenized (3 min) in a stomacher (Interscience, Saint Nom la Bretèche, France). Cellular suspensions were then properly diluted and enumerated by the drop count technique (Miles *et al*., 1938), in triplicate, on selective agar plates except for the samples inoculated with *L. innocua* at 4 log CFU g^{-1} that were enumerated by spread plate. When *Listeria* cells were lower than 2 log CFU g^{-1} in Alheira, the detection was performed according to the International Organization for Standardization (ISO 11290-1:1996/Amd.1:2004). LAB counts were performed on MRS incubated at 30ºC for 72 h under microaerophilic conditions and *L. innocua* was enumerated on PALCAM Agar (Merck, Germany) at 30ºC for 72 h. The colony-forming units per gram (CFU g^{-1}) were calculated as mean value of the mean value from results obtained from each Alheira and transformed to log values prior to further analysis.

Statistical analysis

Microbial reduction was expressed in terms of the difference between the logarithm of the initial number of microorganism before the treatment and logarithm of the number of microorganisms surviving the treatment. During the storage period, the microbial reduction was considered as the difference between the logarithm of the number of microorganism at time 0 and logarithm of the number of microorganisms surviving at each sampling time. One-way analysis of variance (ANOVA), using SPSS® 17.0 for Windows (1994), was carried out to determinate significant differences within and between groups. Tukey's test was applied to compare the mean values. Statistical significance was set at $P < 0.05$.

Results and Discussion

The objective of the present study was to evaluate the effect of combine biopreservation methods (in situ and ex situ bacteriocin production) with a pressure treatment to control *L. innocua* in a traditional Portuguese fermented meat sausage (Alheira). Preliminary pressure inactivation studies (300-600 MPa, 10ºC, 5-15 min) for both pediococci strains (*P. acidilactici* HA-6111-2 and HA-2485-3) in Alheira were assessed (data not shown). For pressures ≥ 400 MPa, both strains were differently affected: the bacteriocinogenic *P. acidilactici* HA-6111-2 started to be inactivated at 400 MPa and demonstrated to be more pressure sensitive than *P. acidilactici* HA-2485-3, the strain used as a negative control for bacteriocin production (data not shown). Sensitivity towards pressure by LAB strains has already been reported (Carlez *et al.*, 1994; Krasowska *et al*., 2005) and it is well accepted that this sensitivity is strain and pressure dependent (Marco *et al*., 2005; Slongo *et al*., 2009). Recently, Castro *et al*. (2015) pressurized *P. acidilactici* HA-6111-2 cells at the stationary growth phase (MRS, pH 6.3) and observed that cells started to be inactivated for pressures higher than 300 MPa, and the production of its bacteriocin also decreased. Therefore, the pressure treatment chosen to treat Alheiras was 300 MPa for 5 min (10ºC) in combination with the pediocin bacHA-6111-2 (in and ex situ production) and the results will be discussed

below.

The recovery of *Listeria* during storage is a critical issue for high pressure processed foods since, and from the point of view of processors, when food safety is overestimated it can cause serious problems to public health. Prolonged shelf life may contribute to the survival and exponential growth of *L. innocua* in contaminated Alheiras. Survival of *L. innocua* was evaluated just after the inoculation, before and immediately after HPP treatment, alone or combined with pediocin bacHA-6111-2, at different cell concentrations (4, 6 and 8 log CFU g^{-1}). These concentrations were selected according to Ferreira *et al.* (2007) where *L. monocytogenes* was present in Alheiras, up to 5.5 log CFU g^{-1}. No significant ($P > 0.05$) changes were observed in *L. innocua* inoculated alone in Alheiras when compared to the initial values, with the exception of samples with ca. 6 log CFU g^{-1} before pressurization (Figure 1). Concentrations of pediococci strains cells after the inoculation, with and without *L. innocua*, was within the range of 8.2 to 9.3 log CFU g^{-1}, with the exception of *P. acidilactici* HA-6111-2 inoculated alone (data not shown).

Under refrigerated storage, growth of *L. innocua* cells inoculated at different concentrations, in non- and pressure treated Alheiras, was followed for 60 days and data is also shown in Figure 1. After 14 days of storage, pressurized samples inoculated with *L. innocua* at ca. 4 log CFU g^{-1}, representing a post-processing contamination, showed a decrease minor than the detection limit (2 log CFU g^{-1}) (Figure 1), but still remained detectable by the ISO 11290-1:1996/Amd.1:2004. For the other pressurized samples, and after a lag period of 7 to 21 days, *L. innocua* cells rapidly increased to 8.2 to 9.3 log CFU g^{-1}. Significant lag phases of *L. monocytogenes* were also reported for pressurized (250 MPa, 9ºC, 20 min) chilled, cold-smoked salmon (Lakshmanan *et al.*, 2004). It should also be mentioned that during the storage period, no cellular counts on TSAYE, MRS and PALCAM were obtained for sterile samples and for Alheiras with bacteriocin bacHA-6111-2, in both control and pressure treated.

Since injury induced by the pressure may be repairable and cells could grow after repairing the site of injury during storage, two different biopreservation methods were then applied: in situ and ex situ bacteriocin production. Figure 2 revealed the effect of *in situ* production of bacteriocin from *P. acidilactici* HA-6111-2 alone and when applied in combination with pressure to control *L. innocua*, at different concentrations. From the inoculation until immediate pressurization, a reduction ($P < 0.05$) in *L. innocua* counts in Alheiras with *P. acidilactici* HA-6111-2, from 1.4 to 1.7 log CFU g^{-1}, was observed; a higher reduction was registered for *L. innocua* cells at ca. 8 log CFU g^{-1}. During the first days of storage, there was even a slight ($P < 0.05$) increase in pressure treated samples, followed by a steady decrease until the end of the study. The initial *L. innocua* increase could be related to the fact that pressurized *P. acidilactici* HA-6111-1 cells were not yet producing bacteriocin or in enough amounts to control *L. innocua* growth. Previously, when Castro *et al.* (2015) studied the kinetic growth at optimal temperature conditions of pressurized *P. acidilactici* HA-6111-2 cells at pressures ≥ 300 MPa, besides a decrease in the maximum level of bacteriocin bacHA-6111-2 production, a presence of a lag production period was also obtained.

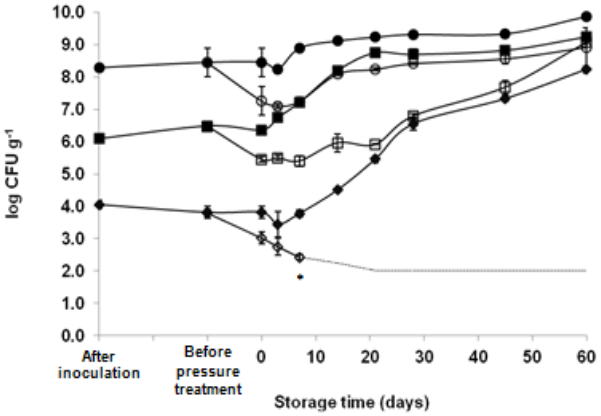

Figure 1. Growth of *L. innocua* cells inoculated at different concentrations, in Alheira paste, under refrigerated storage. (●, o) *L. innocua* (ca. 8 log CFU g^{-1}), non-treated and pressure treated, respectively; (■ , □) *L. innocua* (ca. 6 log CFU g^{-1}) non-treated and pressure treated, respectively; (♦ , ◊) *L. innocua* (ca. 4 log CFU g^{-1}), non-treated and pressure treated, respectively. *From this storage period, values were below the detection limit (2 log CFU g^{-1}). Each data point represents the average calculated from 2 independent assays and the error bars represent the standard deviation.

As it can be seen in Figure 2, the presence of *P. acidilactici* HA-6111-2 in Alheiras with *L. innocua* induced a bacteriostatic effect on the growth of *Listeria* cells up to 21-45 days, and this effect was more obvious for lower initial inoculum level. After 60 days, inactivation values of 0.9 to > 1.5 log CFU g^{-1} were observed when compared to time 0. For similar samples, but submitted to pressure, higher inactivation values were obtained. Combined pressure with in situ pediocin production maintained the counts of *L. innocua*, inoculated at ca. 4 log CFU g^{-1}, under the detection limit (< 2 log CFU g^{-1}). Linking Figures 1 and 2, a synergistic effect between in situ production of pediocin bacHA-6111-2 and HPP, mainly in Alheiras with higher *L. innocua* concentrations, in the last days of storage, was

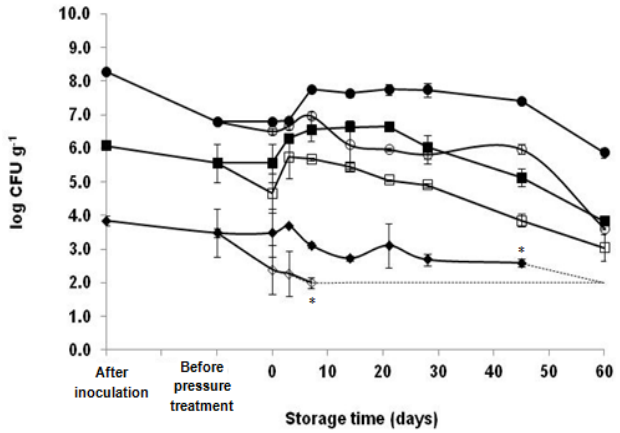

Figure 2. Growth of *L. innocua* cells, at different concentrations, with *P. acidilactici* HA-6111-2 (ca. 8 log CFU g^{-1}) in Alheira paste, under refrigerated storage. (● , o) *L. innocua* (ca. 8 log CFU g^{-1}), non-treated and pressure treated, respectively; (■ , □) *L. innocua* (ca. 6 log CFU g^{-1}), non-treated and pressure treated, respectively; (♦ , ◊) L. innocua (ca. 4 log CFU g^{-1}), non-treated and pressure treated, respectively. *From this storage period, values were below the detection limit (2 log CFU g^{-1}) were obtained. Each data point represents the average calculated from 2 independent assays and the error bars represent the standard deviation.

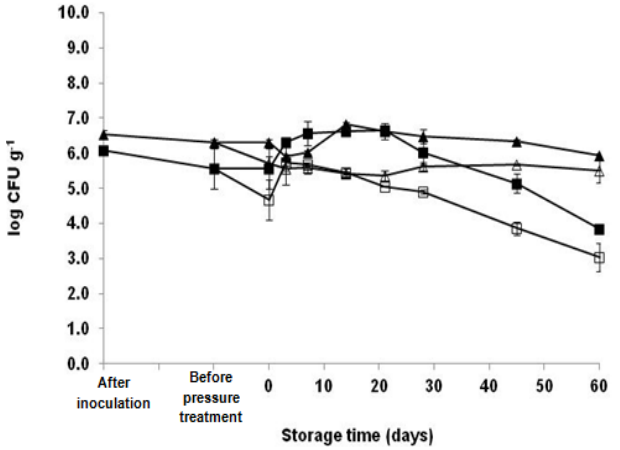

Figure 3. Growth of *L. innocua* cells (ca. 6 log CFU g^{-1}), with pediococci strains in Alheira paste, under refrigerated storage. (■ , □) *P. acidilactici* HA-611-2 (ca. 8 log CFU g^{-1}), non-treated and pressure treated, respectively; (▲ , Δ) *P. acidilactici* HA-2485-3 (ca. 8 log CFU g^{-1}), non-treated and pressure treated, respectively. Each data represent the average calculated from 2 independent assays and the error bars represent the standard deviation.

observed.

Growth of LAB strains on non-pressurized and pressurized Alheiras, in the absence / presence of *L. innocua*, was also monitored during refrigerated storage (data not shown). During the first days, both pressure treated Alheiras inoculated with LAB strain HA-2485-3 or HA-6111-2 showed a higher decreased in their survival rate through storage when compared with non-pressure treated Alheiras (data not shown). However, in less than one month, the cells in these samples quickly recovered to values close to the initial levels. It has previously been demonstrated that the growth rate of pediococci in pressurized meat products under refrigerated storage varies among the strains. Marcos et al. (2005) reported a rapid recovery of endogenous LAB viability after pressurization at 300 MPa (17ºC, 10 min) of low-acid meat sausages. And Garriga *et al*. (2002) encountered LAB recovery to levels close to initial values during chilled storage of meat homogenates processed at higher pressure treatments (400 MPa, 17°C, 10 min).

The effect of LAB strains, *P. acidilactici* HA-6111-2 and HA-2485-3 (used as a negative control regarding the bacteriocin production), with *L. innocua* in pressurized and non-pressurized Alheiras was compared and analysed, and presented in Figure 3. The results only resume the counts of *L. innocua* inoculated at ca. 6 log CFU g^{-1}, since no significant differences ($P > 0.05$) were observed for the other two cells concentration in the presence of *P. acidilactici* HA-2485-3. Up to 21 days of storage, non-pressurized samples presented similar ($P > 0.05$) pattern regarding *L. innocua* cell counts in the presence of P. acidilactici HA-6111-2 or *P. acidilactici* HA-2485-3, while between the analogous pressurized samples significant differences ($P < 0.05$) were observed 7 days earlier. The presence of the bacteriocinogenic LAB strain decreased *L. innocua* cells in Alheiras, with greater importance in the ones pressure treated.

Finally, the influence of *ex situ* bacteriocin bacHA-6111-2 alone and combined with HPP to control *L. innocua,* at different cell concentrations, was also studied and data is shown in Figure 4. When pediocin bacHA-6111-2 (ca. 320 AU g^{-1}) was added to Alheiras, and counts were performed after 5 min, there was a declination on *Listeria* cells from 0.4 to 1.2 log CFU g^{-1}. Similar *L. monocytogenes* reductions (0.5 to 2.2 log) were already reported by Nielsen *et al.* (1990), after a 2 min contact with a bacteriocin from a commercial *P. acidilactici*. This class of bacteriocins tends to be adsorbed to the cytoplasmic membrane of the target cell through the presence of a cell surface receptor or lipid vesicles in *Listeria* which lead to cell lysis. The effect of bacteriocin on the survival of *Listeria* should then be evaluated immediately after its addition. Such effect associated with the impact that several food components might have on microorganisms during a certain period of time before pressurization, is often omitted or even hidden by the processing treatment (Kalchayanand *et al.*, 1994; Garriga *et al.,* 2002).

From the addition of pediocin bacHA-6111-2

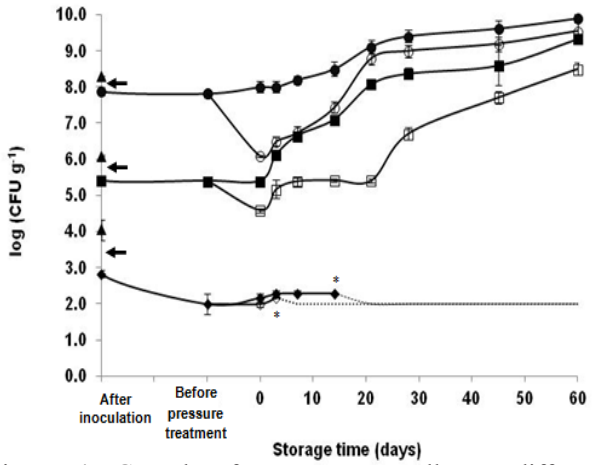

Figure 4. Growth of *L. innocua* cells, at different concentrations (▲), with bacHA-6111-2 (320 AU g^{-1}) in Alheira paste (←), under refrigerated storage.(● , o) *L. innocua* (ca. 8 log CFU g^{-1}), non-treated and pressure treated, respectively; (■ , □) *L. innocua* (ca. 6 log CFU g^{-1}), non-treated and pressure treated, respectively; (♦ , ◊) *L. innocua* (ca. 4 log CFU g^{-1}), non-treated and pressure treated, respectively. *From this storage period, values were below the detection limit (2 log CFU g^{-1}). Each data point represents the average calculated from 2 independent assays and the error bars represent the standard deviation.

until pressurization, no significant ($P > 0.05$) decrease in *L. innocua* cells was further obtained in the presence of pediocin bacHA-6111-2, with the exception of the lowest bacteria cell (Figure 4). The effect of bacteriocin on *L. innocua* cells continues to be dependent on the inocula concentration. Samples with higher *L. innocua* cells reached values of 8 to 9 log CFU g^{-1} by the end of storage. The antilisterial effect was greater at low cell levels, with a reduction > 2 log CFU g^{-1} was observed. When the effect of in and ex situ production of bacteriocin on *L. innocua* at elevated concentrations is compared (Figures 2 and 4), in situ production has shown to have a better efficacy during long periods of storage. Possibly, the amount of bactericion initially added to Alheiras was not enough to control the growth of *L. innocua* or it could have lost its antilisterial activity in the paste due to its chemical composition (e.g., high amount of fat). After the addition of pediocin from a *P. acidilactici* strain H to minced meat, Dickson *et al.* (1999) determined its residual activity, and reported that it immediately reduced to 75 % of its initial activity and, with further storage at 7ºC, the value felt to levels below 1% after 15 days.

For pressurized Alheiras with pediocin bacHA-6111-2 and in the case of 4 log CFU g^{-1} of *L. innocua,* bacteriocin bound to the cell membrane of *L. innocua* could have increased its susceptibility towards pressure inactivation, and achieved a synergistic bacterial kill, an effect similar to the one described by ter Steeg *et al.* (1999). According to these authors, bacteriocins bound to the cell membrane of *L. plantarum* increased its susceptibility to inactivation by mild pressures (100-300 MPa) at low temperatures, and achieved a synergistic bacterial kill (> 6 log reduction) for 200 MPa (room temperature), at pH 7. At the same time, *L. innocua* cells sublethally injured by pressure could have decreased the resistance to the pediocin bacHA-6111-2 still present in the pastes, and as consequence, the bacteria counts were maintained under the detection limit. Kalchayanand *et al.* (1994) have also concluded that high pressure induced sublethal injury to bacterial cells, which became more sensitive to pediocin AcH and nisin.

Overall, the effect of combined treatments versus each treatment alone showed higher reduction rates emphasizing the synergism between the in situ production of bacteriocin and pressure. At the end of storage, the applied pressure treatment alone (Figure 1) or even combined with *ex situ* pediocin (Figure 4) only reduced ca. 2 log CFU g^{-1} for the lowest *L. innocua* cells concentration when compared to the corresponding control; no reduction was obtained for the highest *L. innocua* concentrations. But when in combination with in situ pediocin (Figure 2), a decrease of 1.9 to 4.7 log CFU g^{-1} was observed.

Conclusion

A synergism between the applied biopreservation methods and pressure treatment was shown. During the first days of storage, and for high levels of *L. innocua,* a bacteriostatic effect was observed for both in and *ex situ* production of pediocin bacHA-6111-2 in combination with pressure. But pressurized Alheiras with *in situ* production was considered a better treatment for prolonged storage. For *L. innocua* cell concentrations close to levels potentially arising by natural post-contamination in meat products (≤ 4 log CFU g^{-1}), the levels were reduced and kept under the detection limit (< 2 log CFU g^{-1}) within the first days of storage when pediocin bacHA-6111-2 (in and *ex situ*) was combined with pressure. This study demonstrated the potential of using the combination of natural antimicrobial compounds (pediocin bacHA-6111-2) with mild pressure (300 MPa, 5 min) treatments to control effectively *L. innocua* in fermented meat products such as Alheiras.

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