Food Bioscience 19 (2017) 65–72

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Biopreservation strategies in combination with mild high pressure treatments in traditional Portuguese ready-to-eat meat sausage

S.M. Castro[a,b], M. Kolomeytseva[a], R. Casquete[a,c], J. Silva[a], R. Queirós[b], J.A. Saraiva[b], P. Teixeira[a,*]

[a] CBQF-Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal
[b] QOPNA, Departamento de Química, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
[c] Nutrición y Bromatología, Escuela de Ingenierías Agrarias, Universidad de Extremadura, Badajoz, Spain

ARTICLE INFO

Keywords:
High pressure
Biopreservation
Listeria innocua
Pediococcus acidilactici
Bacteriocin
Meat sausage

ABSTRACT

Mild pressure (300 MPa, 5 min, 10 °C) was combined with lactic acid bacteria (*Pediococcus acidilactici*, HA-6111-2) and its bacteriocin (bacHA-6111-2) to treat traditional Portuguese ready-to-eat meat sausage (*Chouriço de carne*) and the effect on the survival of *Listeria innocua* was evaluated. Five batches of sausage slices were prepared: i) non-inoculated; ii) inoculated with *L. innocua*; iii) inoculated with *P. acidilactici*; iv) inoculated with *L. innocua* and *P. acidilactici*; and v) inoculated with *L. innocua* and bacteriocin. Pressure had no impact on the growth of *P. acidilactici* cells. In non pressurized samples inoculated with *L. innocua*, cells were inactivated by the traditional hurdles (e.g., salt, spices), and the reduction was higher when pressure alone was applied. No significant differences ($P > 0.05$) were observed for *L. innocua* co-inoculated with *P. acidilactici* alone or in combination with pressure, but when inoculated with the bacteriocin, an immediate and further decrease was obtained by pressurization. During refrigerated storage, the highest inactivation rate was obtained for pressurized slices inoculated with *L. innocua* in the presence of bacteriocin. The use of bacteriocins can effectively contribute to food safety, especially when integrated into hurdle concepts. This treatment strengthens the traditional hurdles applied to slices of *Chouriço de Carne*.

1. Introduction

Traditional dry fermented sausages rely on natural contamination by environmental microbiota to develop its organoleptic attributes. This contamination occurs during slaughtering and increases during manufacturing. Several authors have described the importance of the natural microbiota during the fermentation process, namely lactic acid bacteria (LAB) and *Micrococcaceae*, responsible for inhibition of undesirable microbial growth (either by decreasing the pH or by production of antimicrobial compounds, such as bacteriocins), and development of particular colour and flavour (Ferreira, Barbosa, Silva, Gibbs, Hogg, & Teixeira, 2009; Martín, Colín, Aranda, Benito, & Córdoba, 2007).

Together with the high salt level, the presence of organic acids and nitrites, the progressive decrease of water activity (a_w), competition with endogenous microbiota, addition of spices, herbs and smoke during the fermentation process are generally the barriers considered as traditional hurdles against the pathogenic bacteria (Työppönen, Markkula, Petaja, Suihko, & Mattila-Sandholm, 2003). However, there is still a probability that some pathogenic organisms, including L. *monocytogenes*, could overcome these hurdles imposed during processing. The presence of pathogens in fermented sausages has often been reported (Ferreira et al., 2009; Thévenot, Delignette-Muller, Christieans, & Vernozy-Rozand, 2005; Työppönen et al., 2003). EC Regulation 2073-2005 requires that food products are negative for L. *monocytogenes* in 25 g samples whilst under the control of the manufacturer and $< 10^2$ CFU g^{-1} in the marketplace. Moreover, contamination of ready-to-eat (RTE) meat products often occurs mainly due to post-process contamination, i.e. during slicing or packaging (Ferreira et al., 2009; Kouakou, Ghalfi, Destain, Dubois-Dauphin, Evrard, & Thonart, 2009). The most frequently applied hurdles applied to these products include nitrites, vacuum packaging and refrigerated storage. Several studies have addressed the use of HPP treatment as an in-package final process step to eliminate post-cooking contamination of RTE meat products and thus increase their safety and shelf-life, and were reviewed by Garriga and Aymerich (2009). To ensure safety

* Corresponding author.
E-mail address: pcteixeira@porto.ucp.pt (P. Teixeira).

http://dx.doi.org/10.1016/j.fbio.2017.05.008
Received 16 June 2016; Received in revised form 5 May 2017; Accepted 26 May 2017
Available online 08 June 2017

considerations but, at the same time, lowering the amount of chemical preservatives whilst conserving the desirable characteristics of most foods (Altuntas, Kocan, Cosansu, Ayhan, Juneja, & Materon, 2012; Campos, Castro, Rivas, & Schelegueda, 2013; Ferreira, Almeida, Delgadillo, Saraiva, & Cunha, 2016) high pressure processing (HPP) can be considered as an alternative to be included in hurdle technology thus appealing "more aware" consumer market.

The application of bacteriocinogenic LAB strains such as pediococci or their metabolites when combined with other chemical compounds or physical processes can make use of synergies to increase microbial inactivation, without altering nutritional value and organoleptic properties of food (Gálvez, Abriouel, López, & Ben Omar, 2007). Two main strategies for the application of bacteriocins in the preservation of food may be considered: the inoculation of a bacteriocinogenic culture as a starter or protective culture (in situ production) and the addition of purified or semi-purified bacteriocin as a food preservative (ex situ production). But the application of *ex situ* bacteriocins in meat systems has been reported to have limitations associated with interaction with phospholipids, low solubility and inactivation by meat endogenous enzymes (Campos, Castro, Rivas, & Schelegueda, 2013). In situ production of bacteriocins in combination with HPP has been shown to be a feasible procedure to improve safety of certain food products, e.g., cheese (Rodriguez, Arques, Nuñez, Gaya, & Medina, 2005), but in other products the performance of the bacteriocinogenic cultures might be negatively influenced by the endogenous microbiota, the food matrix and the technology itself (Gálvez et al., 2007). A number of factors must be considered when choosing the bacteriocinogenic culture (e.g., the ability to produce bacteriocin under the studied conditions, diffusion in the food matrix, binding to other food components, lack of any pathogenic traits, absence of any acquired antibiotic resistance, among others (Altuntas et al., 2012; Campos et al., 2013).

The combination of antimicrobial agents and post-package pasteurization using HPP seems to be the most effective and economically feasible intervention technology (Jiang & Xiong, 2015). The objective of this work was to assess the effect of two biopreservation strategies (in situ and ex situ bacteriocin production) combined with HPP, used in the commercial pressure range (400–600 MPa), on the survival of *L. innocua* during refrigerated storage of slices of *Chouriço de carne*, a traditional Portuguese RTE meat product. This study is the first report on the bactericidal effect of *P. acidilactici* strain (HA-6111-2) with both in situ and ex situ production of bacteriocin in combination with mild pressure processing as hurdles to enhance the safety vacuum packed RTE *Chouriço de carne* slices.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Listeria innocua 2030c (Escola Superior de Biotecnologia, Porto, Portugal) was grown on tryptone soya agar with yeast extract 0.6% (w v^{-1}) (TSA-YE, Lab M, Bury, UK) and subcultured in tryptone soya broth with yeast extract (TSB-YE, Lab M), at 37 °C for 24 h. *Pediococcus acidilactici* HA-6111-2, a strain with antilisterial activity (Albano, Oliveira, Aroso, Cubero, Hogg, & Teixeira, 2007), was cultured on de Man, Rogosa and Sharpe (MRS) agar (Lab M) and subcultured twice in MRS broth (Lab M), at 37 °C, for 18–24 h. Both bacterial cells were harvested by centrifugation (6000*g*, 10 min, 4 °C; Rotina 35R, Hettich, Germany) under aseptic conditions, washed twice in sterile quarter-strengh Ringer's solution (Lab M) and re-suspended to ~ 10^9 CFU ml^{-1}.

2.2. Bacteriocin crude extract

The bacteriocin crude extract was obtained from the supernatant during the harvesting of *P. acidilactici* cells as mentioned in Section 2.1. The culture supernatant was adjusted to pH 6 by the addition of NaOH (1 mol l^{-1}) and heated at 85 °C (10 min). Briefly, a doubling dilution series was made of the cell-free culture supernatant, containing the bacteriocin (bacHA-6111-2). An aliquot of 10 ml of each dilution was spotted onto an agar plate (0.7% w v^{-1} agar) seeded with ~ 10^6 cells/ml of *L. innocua* 2030c (target organism), and further incubated at 37 °C. Antimicrobial activity was expressed as arbitrary units (AU) per ml (AU ml^{-1}). One AU is defined as the reciprocal of the highest dilution showing a clear zone of growth inhibition (Van Reenen, Dicks, & Chikindas, 1998). The initial bacteriocin activity in the supernatant was 6400 AU ml^{-1}.

2.3. Physico-chemical parameters of ready-to-eat meat slices

Vacuum pre-packed commercial RTE slices of *Chouriço de carne* (~ 1 g/slice) were kindly supplied by Minhofumeiro (Ponte de Lima, Portugal), brought to the laboratory and kept at 7 °C, until further analysis. Sodium chloride (current method: hot extraction of chloride, followed by precipitation with silver nitrate) and moisture (dry weight determination, until constant weight) contents were determined following the Portuguese Standard NP 1845 (IPQ, 1982) and NP 1614 (IPQ, 2002), respectively. pH was determined directly with a Crison MicropH 2002 pH-meter (Crison, Barcelona, Spain), equipped with an InLab 427 puncture electrode (Mettler Toledo, Columbus, OH, USA). According to the manufacturer's instructions, the water activity (a_w) was measured with a Hygropalm AW1 (Rotronic Instrument Corporation, New York, NY, USA). The total fat and proteins (conversion factor N × 6.25) contents were determined by Soxhlet extraction (with petroleum ether) and the Kjeldhal method, respectively, according to Slack (1997), and values for both nitrites and nitrates were determined by a photometric method (538 nm), according to the ISO Standards 2918 and 3091 (Anonymous, 1975a, 1975b).

2.4. Experimental conditions

2.4.1. Preparation of ready-to-eat meat samples

The slices of *Chouriço de carne* (~ 1 g/slice; diam. ~ 3 cm) were kept for 72 h at 7 °C. Before inoculation, samples were screened for L. *monocytogenes* using the mini-VIDAS method (Anonymous, 1996), an enzyme-linked fluorescent immunoassay done in the automated VIDAS® instrument (bioMérieux, Marcy l'Etoile, France), using an antibody specific for L. *monocytogenes*, and also by direct enumeration according to the International Standard ISO 11290-2 (Anonymous, 1998). The inoculation conditions of the slices were: i) non-inoculated (*S*); ii) inoculated with *L. innocua* (L), iii) inoculated with *P. acidilactici* HA-6111-2 (P), iv) inoculated with *L. innocua* and *P. acidilactici* HA-6111-2 (L + P); and v) inoculated with *L. innocua* and bacteriocin (bacHA-6111-2) (L + B). For each condition, slices were immersed in *L. innocua* and/or *P. acidilactici* inocula (~ 10 ml, 10^9 CFU ml^{-1}), for 2 min. Slices (L + B) were first immersed in *L. innocua* cells, sprayed on both sides with the bacteriocin solution (~ 6400 AU ml^{-1}) and left to dry for 10 min. For each condition, two slices of *Chouriço de carne* were aseptically double packed in low permeability polyamide-polyethylene bags (PA/PE-90, Albipack-Packaging Solutions, Águeda, Portugal), and vacuum-sealed (Culinary, Albipack – Packaging Solutions, Águeda, Portugal). Further, the non-pressurized, to be kept as controls, and pressurized samples had the subscript notation C (S_C, L_C, P_C, L + P_C, and L + B_C) and HPP (S_{HPP}, L_{HPP}, P_{HPP}, L + P_{HPP}, L + B_{HPP}), respectively.

2.4.2. High pressure processing

The pressure treatment was carried out using a hydrostatic press from Unipress Equipment, Model U33 (Warsaw, Poland), with a pressure vessel of 100 ml (35 mm diameter and 100 mm height), surrounded by an external jacket, connected to a thermostatted bath (Huber Compatible Control CC1, Trenton, NJ, USA). The pressure-transmitting fluid was a mixture of propylene glycol and water (60:40). The pressure treatment was set at 300 MPa, with a pressurization rate