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Active packaging containing nisin and high pressure processing as post-processing listericidal treatments for convenience fermented sausages

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ABSTRACT

Listeria monocytogenes was inoculated on the surface of sliced fermented sausages with no added sodium salt. The pathogen was progressively inactivated during the product shelf life (90 days). Antimicrobial packaging of fermented sausages with PVOH films containing nisin induced a more pronounced reduction of *L. monocytogenes* counts during refrigerated storage. HPP alone (600 MPa, 5 min, 12 °C) had no antimicrobial effect against *L. monocytogenes* at the studied conditions. Combination of HPP with antimicrobial packaging did not produce any extra protection against *L. monocytogenes* compared to antimicrobial packaging alone. The lack of effect of HPP on *L. monocytogenes* was attributed to a protective effect exerted by the low water activity of the product and its lactate content. These results reflect that antimicrobial packaging with the inclusion of nisin as a natural antimicrobial could be considered as an effective method to reduce the levels of *L. monocytogenes* in sliced fermented sausages with no added sodium salt.

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1. Introduction

Fermented sausages are generally considered stable and low-risk products as a consequence of a combination of hurdles, whose interaction inactivate or prevent the growth of undesired microorganisms present in the product (Leistner & Gorris, 1995). The development of meat products with reduced or no added sodium salt could alter this sequence of hurdles and could have a negative effect on food safety. Therefore, the development of reduced salt meat products would require changes in product formulation and/or application of post-processing technologies that provide additional hurdles to pathogen growth in order to assure food safety.

Although there have been major gains over the last decade in reducing contamination of ready-to-eat (RTE) meat products, *Listeria monocytogenes* continues to be a major concern for food safety (Batz, Hoffmann, & Morris, 2011). The prevalence of *L. monocytogenes* in European industries manufacturing fermented sausages has been extensively documented both in the product and the equipment (De Cesare, Mioni, & Manfreda, 2007; Martin, Garriga, & Aymerich, 2011; Talon et al., 2007; Thévenot, Delignette-Muller, Christieans, & Vernozy-Rozand, 2005). Investigations of Italian and Spanish industries showed a prevalence of *L. monocytogenes* in fermented sausages of about 15% among the studied samples (De Cesare et al., 2007; Martin et al., 2011). The prevalence of *L. monocytogenes* in fermented sausages, together with the increased number of listeriosis cases (19.1% increase in 2009 in respect to 2008) that present a high case fatality ratio of 16.6% (European Food Safety Authority, 2011) reflect the importance to assure *L. monocytogenes* inactivation in RTE meat products that will not be processed prior to its consumption.

Antimicrobial packaging has been proposed as an alternative to post-packaging operations to improve RTE products safety. The main benefit of applying antimicrobial compounds through packaging rather than direct addition in the food matrix is due to an increased antimicrobial efficiency. The localization of the antimicrobial compound on the surface of the slices, where contamination can occur, together with a lower inactivation by adsorption of the antimicrobial to the food constituents may explain its improved efficiency (Aasen et al., 2003). Natural antimicrobials such as nisin have proved to be effective against microbial growth when added to the food products through packaging systems (Coma, Sebti, Pardon, Deschamps, & Pichavant, 2001; Ercolini et al., 2010; Hereu, Bover-Cid, Garriga, & Aymerich, 2012). Nisin has been shown to be effective in a number of food systems, inhibiting the growth of a wide range of Gram-positive bacteria, including food-borne pathogens such as *L. monocytogenes* (Benkerroum & Sandine, 1988; Brewer, Adams, & Park, 2002; Ukuku & Shelef, 1997).

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High-pressure processing (HPP) improves safety and to extend the shelf life of RTE food products because is capable of inactivating microorganisms and endogenous enzymes, while maintaining nutrients and flavours (Ross, Griffiths, Mittal, & Deeth, 2003). Overall, HPP inflicts lethal and/or sublethal injuries on microorganisms, mainly due to membrane damage (Kalchayanand, Sikes, Dunne, & Ray, 1998). Sublethally injured cells are more susceptible to antimicrobial compounds (Kalchayanand, Sikes, Dunne, & Ray, 1994).

In this context, the aim of the present work was to study the behaviour of *L. monocytogenes* inoculated on sliced fermented sausages with no added sodium salt obtained with the QDS® (Quick-Dry-Slice) system (Arnau, Serra, Comaposada, Gou, & Garriga, 2007) and to assess the combined effect of antimicrobial packaging and high pressure processing (HPP) used as post-processing listericidal treatment.

2. Materials and methods

2.1. Product description

Sliced fermented sausages with no added sodium salt dried with the Quick Dry Slice (QDS®) process were kindly provided by Casademont, S.A. (Bonmatí, Spain). The additives added to the product were: potassium lactate, ascorbic acid, dextrose, lactose, aroma, gluco delta-lactone, species, potassium chloride, tetrapotassium pyrophosphate, colourant (cochineal carmine), potassium nitrite, potassium nitrate. Composition of fermented sausages used in this study is shown in Table 1.

Briefly, lyophilized *Lactobacillus sakei* and *Staphyloccocus carnosus* (Bactoferm F-SC-111, Christian Hansen, Hoersholm, Denmark) were used as starter culture. The sausages were fermented for 48–72 h at 22 °C until the desired pH was reached. After fermentation the sausages were frozen, sliced and dried with the QDS® process, a continuous system based on convective drying of slices (Arnau et al., 2007).

2.2. Nisin solution

A saturated solution of nisin was obtained by dissolving 0.4 g/ml of Nisaplin® (Danisco, Copenhagen, Denmark) in sterile distilled water. After mixing the solution was allowed to rest overnight. The solution was centrifuged for 30 s at 5000 rpm, after centrifugation a three phase solution was obtained. The active fraction of the mixture was recovered after discarding the precipitate and the upper liquid phase.

2.3. Bacteriocin assay

The indicator strains, *L. monocytogenes* CTC1011 (serovar 1/2c), CTC1034 (serovar 4b) and CECT 4031 (serovar 1a) were separately grown overnight in Tryptic Soy Broth with 0.6% yeast extract (TSBYE; Merck, Darmstadt, Germany) at 37 °C and mixed together in equal proportions. Nisin activity was quantified by the agar spot test (Tagg, Dajani, & Wannamaker, 1976). A solid agar base composed of 20 g/l beef extract, 20 g/l glucose, and 15 g/l agar, was used to support soft TSBYE (TSBYE with 7.5 g/l agar) seeded with 20 μl of the overnight cocktail strain of *L. monocytogenes*. Nisin solutions were serially twofold diluted with 50 mM phosphate buffer, pH 6. A 10 μl sample of each dilution was spotted onto soft TSBYE lawn. The plates were incubated overnight at 37 °C. An arbitrary unit (AU) was defined as the highest dilution showing growth inhibition of the indicator lawn, and nisin solution activity was expressed as AU/ml.

The activity of the concentrated solution of nisin was 409,600 AU/ml.

Table 1
Composition of sliced dry-fermented sausages (QDS®) with no added sodium salt.

Protein (%)	20
Fat (%)	20
Moisture (%)	40
Carbohydrates (%)	6
Sodium (%)	0.13
Nitrites (KNO_2, mg/kg)	6
Nitrates (KNO_3, mg/kg)	130
Potassium lactate (g/kg, 77.8% purity)[a]	28.15

[a] Added amount.

2.4. Film manufacturing

Film forming solutions were obtained as suggested by Del Nobile et al. (2003) with some modifications. A 13% (w/v) solution of fully hydrolysed polyvinyl alcohol (Elvanol® 90-50, kindly provided by DuPont™) in distilled water was dissolved for 20 min in an autoclave at 121 °C. After measuring the volume of the film blend, the active solution was obtained by adding 1% of the concentrated solution of nisin (409,600 AU/ml) to obtain a concentration of 450 AU/cm^2. The films were manufactured by pouring 7 ml of the prepared solutions onto sterile polystyrene dishes, and were dried for 10 h under laminar flow in a biological safety cabinet (BIO-IIA; Telstar, Terrassa, Spain). After solvent evaporation, the films were stripped directly from the dishes.

The thickness of the films was measured by means of a Digimatic Micrometer (Mitutoyo, Japan). The value of the film thickness was obtained by averaging 10 measurements. The films obtained had an average thickness of 108 ± 15 μm.

Antimicrobial activity against *L. monocytogenes* of nisin containing films was verified *in vitro* by placing a 1 cm diameter film sample on the surface of solid agar base and TSBYE soft agar plates seeded with an overnight mixture of *L. monocytogenes* as described in the previous section. Agar plates were incubated at 37 °C overnight and antimicrobial activity of films was observed as a zone of inhibition of the indicator strains around the films.

2.5. Sample preparation and high-pressure processing

Fermented sausage slices were inoculated with 5×10^5 CFU/g of a 3-strain cocktail of *L. monocytogenes* (CTC1011, CTC1034 and CECT 4031). Fermented sausage slices were placed between two films and packed under vacuum in polyamide–polyethylene bags (Sacoliva, Castellar del Vallès, Spain). Two independent batches were prepared: fermented sausage slices packed with control PVOH films (C), and slices packed with PVOH films containing nisin (N).

Within each batch, half of the samples were kept as non-treated controls (NT) and half were pressurized (HPP) at 600 MPa for 5 min at an initial fluid temperature of 12 °C. HPP was carried out in an industrial hydrostatic pressurisation unit (Wave 6000/120 l, NC Hyperbaric, Burgos, Spain). The come up time was 181 s and the release was almost immediate (<6 s).

Vacuum packed samples were stored at 4 °C for 7 days, trying to reproduce storage conditions in the manufacturer facilities. Afterwards samples were stored at 12 °C until the end of the shelf life 90 days, trying to reproduce the worst case scenario of storage conditions in consumers' refrigerators.

The experiment was replicated in two independent trials.

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