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

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Abstract

L. 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, 12ºC) 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 *Listeria monocytogenes* in sliced fermented sausages with no added sodium salt.

Keywords: fermented sausages, antimicrobial packaging, nisin, high pressure processing, L. monocytogenes

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 (Thévenot et al., 2005, Talon et al, 2007, De Cesare et al 2007, Martin et al., 2011). 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, Markussen, Moretro, Katla, Axelsson & Naterstad, 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 foodborne pathogens such as *L. monocytogenes* (Benkerroum & Sandine, 1988; Brewer, Adams & Park, 2002; Ukuku & Shelef, 1997).

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. (Sant Gregori, Spain). The additives added to the product were: potassium lactate, ascorbic acid, dextrose, lactose, aroma, gluco delta-lactone, species, potassium chloride, tetrapotassium pyrophosphate, colorant (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 22ºC 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 5,000 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 50mM 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.

2.4. Film manufacturing

Film forming solutions were obtained as suggested by Del Nobile, Piergiovanni, Buonocore, Fava, Puglisi & Nicolais (2003) with some modifications. A 13% (w/v) solution of fully hydrolyzed polyvinyl alcohol (Elvanol® 90-50, kindly provided by DuPont^{TM}) 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 10h 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 $5x10^5$ 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.

2.5. Physico-chemical analysis

The pH was measured directly in the samples using a Crison penetration 52-32 electrode connected to a Crison Basic 20 pH-meter (Crison Instruments S.A., Alella, Spain). The mean of three measurements was recorded for each sample. Water activity was measured with a water activity meter AquaLab™ Series 3 (Decagon Devices, Inc., Pullman, WA, USA).

Nitrate and nitrite contents were evaluated with a segmented continuous-flow Autoanalyzer II sampler (Technicon Ltd. Dublin, Ireland) by methods US-230-72A, as recommended by the manufacturer.

Three different samples were analysed at each sampling time.

2.6. Microbiological analysis

Sampling was performed at days 0, 1 (after HPP), 7, 14, 30, 60, and 90 during the shelf life of sliced fermented sausages. At each selected time, 25 g of fermented sausages were 10-fold diluted in sterile LEB broth (Oxoid, Hampshire, UK). The solution was homogenized for 1 min in a Masticator (IUL S.A., Barcelona, Spain). After appropriate dilutions, enumeration of *Listeria monocytogenes* was performed by spread plating on Chromogenic *Listeria* agar (Oxoid Ltd., Basingstoke, England) incubated at 37 °C for 48-72 h. To improve the detection limit to 4 cfu/g, 2.5 ml of the 1/10 dilution was spread on a 14 cm diameter plate. Lactic Acid Bacteria (LAB) and *Enterobacteriaceae* enumeration was done by plating in MRS agar (Merck) incubated in anaerobiosis at 30°C for 72 h and VRBD agar (Merck) at 30 °C for 24 h, respectively.

Three different samples were analysed at each sampling time.

2.8. Statistical analysis

Data were subjected to analysis of variance using the general linear model procedure from the SAS statistical package (SAS System for Windows, Release 8.2, SAS Institute, Cary, NC, USA).

The model included batch, storage time, and their interaction as fixed effects. The trial was included in the model as a block effect. Differences between effects were assessed by the Tukey test ($P<0.05$).

3. Results and discussion

Physico-chemical characteristics

Twenty-four hours after packaging, the water activity (a_w) of the studied fermented sausages was 0.885 ± 0.005 and the pH was 5.61 ±0.02. According to these values, *L. monocytogenes* would not be able to grow in this product type as its growth threshold is around a water activity of 0.93 (ICMSF, 1996). However, the pathogen would be able to survive during the product shelf-life, if post-processing contamination occurred.

Table 2 shows the a_w and pH values of fermented sausages at days 1, 15 and 90 of refrigerated storage. As expected, no important changes on a_w or pH values were observed throughout storage.

The studied treatments (active packaging and HPP) had no or little effect on the a_w and pH values of fermented sausages (Table 2). Only HPP produced a significant increase of

pH values in fermented sausages packed with nisin containing films on days 1 and 15 of storage which agrees with pressure induced increases of pH values observed in raw meat (Macfarlane, McKenzie & Turner, 1982; Macfarlane, 1985; McArdle, Marcos, Kerry & Mullen, 2010). It has been attributed to a decrease in available acidic groups in the meat as a result of conformational changes associated with protein denaturation (Mandava, Fernandez & Juillerat, 1994). Nevertheless, the changes induced during fermentation and curing processes lead to protein denaturation and product stabilisation and would reduce pressure induced changes in cured products. No further differences on pH values among batches were observed ($p>0.05$).

On the other hand, no differences on the a_w values of fermented sausages were observed among batches throughout storage ($p>0.05$).

L. monocytogenes behaviour

Antimicrobial activity of polyvinyl alcohol films was determined *in vitro* on a TSBYE lawn seeded with *L. monocytogenes*. Control PVOH films showed no antimicrobial activity, while nisin containing films showed clear inhibition zones.

L. monocytogenes was inoculated to levels of $5x10^5$ cfu/g on the surface of the fermented sausages. The pathogen was inactivated to some extent by all studied treatments during the product shelf life (Figure 1). Control non-treated samples (C NT) showed a decrease of 2.23 log cfu/g of the pathogen at the end of the study, suggesting that the physico-chemical characteristics of the product favoured a reduction of the population of *L. monocytogenes* throughout storage in this product with no added sodium salt. The low water activity, as well as the presence of nitrite in the formulation could have favoured the reduction of *L. monocytogenes* at the studied conditions (Duffy, Vanderlinde & Grau, 1994; Junttila, Hirn, Hill & Nurmi, 1989; Marcos, Aymerich & Garriga, 2005). Moreover, the content of lactate in the fermented sausage formulation (21.9 g/kg) could have exerted an antimicrobial effect against *L. monocytogenes*. Although sodium lactate is quite effective lowering the a_w of food products, it has been demonstrated that its antimicrobial activity is mainly due to a "specific solute (lactate ion) effect" rather than to lowering the a_w (Chen & Shelef, 1992; Houtsma, Kant-Muermans, Rombouts & Zwietering, 1996).

Antimicrobial packaging of fermented sausages with PVOH films containing nisin (N NT) induced a more pronounced reduction of *L. monocytogenes* counts during the 90

days of refrigerated storage (Figure 1). N NT samples showed counts 1.4 log cfu/g lower than C NT at the end of the fermented sausages shelf life. Similarly, Hereu, Bover-Cid, Garriga & Aymerich (2012) observed final concentrations of *L. monocytogenes* 1 log unit lower than the control batch in dry-cured hams (a_W of 0.88) packed with PVOH films containing a concentration of nisin of 200 AU/cm^2. In another study, antimicrobial films of polyamide/polyethylene embedded with 200 AU/cm^2 of nisin and 1.8% sodium lactate induced a 1.9 log decrease of *L. monocytogenes* population in cooked ham (Jofré, Garriga & Aymerich, 2007). Although the final result would be similar to the present study, the different nature of the meat product that was cooked ham which is able to support the growth of the pathogen resulted in a very different behaviour of the pathogen during refrigerated storage. The effectiveness of antimicrobial packaging is dependent on the type of food packed, the film forming polymer and the type and concentration of antimicrobial that will determine the release rate and therefore the antimicrobial efficiency.

Fermented sausages were pressurised for 5 min at 600 MPa. After HPP (day 1), no extra inactivation of *L. monocytogenes* was observed in C HPP compared to the other treatments (Figure 1). Actually, no differences in *L. monocytogenes* counts ($p>0.05$) were observed between C NT and C HPP samples throughout storage, suggesting that HPP alone had no listericidal effect at the studied conditions.

Considering the proved efficiency of HPP to inactivate *L. monocytogenes*, these results are concerning from a safety point of view. It has been observed that the inactivation rates of HPP are strongly dependent on the *L. monocytogenes* strains tested (Patterson, Mackle & Linton, 2011; Youart, Huang, Stewart, Kalinowski & Legan, 2010). However, the studied strains have proved to be sensitive to HPP (600MPa, 5 min, 13ºC) in previous studies in dry-cured ham (Stollewerk, Jofré, Comaposada, Arnau & Garriga, 2012). Therefore, it seems that the lack of effect of HPP on *L. monocytogenes* would be more related to a protective effect exerted by the food composition. The composition of the food matrix has proved to have a great influence on the lethality of a pressure treatment. Specifically, the a_w is a food property that strongly influences the inhibitory effect of HHP. Low values of a_w protect microorganisms against pressure induced inactivation (Smelt, 1998). The influence of a_w on the antilisterial effects of HPP became evident in a study by Hereu et al. (2012) who reported differences of 2 log cfu/g in the inactivation of *L. monocytogenes* present in two types of dry-cured ham with significant differences on a_w values (0.92 and 0.88). Another factor influencing HPP

efficiency is the presence of lactate in the product formulation. The protective effect of lactate is in part related to its capacity of lowering the a_w of the product. However, the mode of action is still not fully understood, as it has proved to have a protective effect against pressure induced inactivation of *L. monocytogenes* even in high water activity products such as cooked ham (Marcos, Jofré, Aymerich, Monfort & Garriga, 2008).

Sliced fermented sausages packed with nisin containing films and submitted to HPP (N HPP) experienced a reduction of *L. monocytogenes* counts of 4.57 log cfu/g throughout refrigerated storage. At the end of the product shelf life, *L. monocytogenes* levels were 2 log units lower than in the control batch (C NT). However, combination of HPP with antimicrobial packaging (N HPP) did not produce any extra protection against *L. monocytogenes* compared to antimicrobial packaging alone (N NT, Figure 1). These results do not agree with previous observations that reported a synergism between HPP and bacteriocin inactivation of *L. monocytogenes* (Arqués, Rodriguez, Gaya, Medina & Nuñez, 2005; Kalchayanand, Hanlin & Ray, 1992; Marcos et al., 2008). Sublethal injuries would facilitate the access of nisin to the cytoplasm membrane as a result of cell wall permeabilisation (ter Steeg, Hellemons & Kok, 1999). From these results it seems that the protective effect against HPP exerted by the low a_w and the presence of lactate would have prevented not only lethality against *L. monocytogenes* but also sublethal injuries.

Enterobacteriaceae behaviour

The levels of *Enterobacteriaceae* in meat products are an indicative of improper hygienic conditions. The control of *Enterobacteriaceae* growth in fermented sausages is important to prevent quality defects such as the formation of off-flavours and the production of biogenic amines, such as diamines putrescine and cadaverine (Garriga, Hugas, Gou, Aymerich, Arnau & Monfort, 1996; Maijala, Eerola, Lievonen, Hill & Hirvi, 1995; Suzzi & Gardini, 2003).

The evolution of *Enterobacteriaceae* population during refrigerated storage of fermented sausages is shown in Figure 2. Initial levels of *Enterobacteriaceae* were 1.54 ± 0.23 log cfu/g.

Non-pressurised samples showed a significant decrease from initial levels from day 60 in C NT and day 30 in N NT ($p<0.01$). It is well known that *Enterobacteriaceae* hardly grow in the hostile environmental conditions of low water activity, high salinity, and

low pH values created in fermented sausages (Lizaso, Chasco & Beriain, 1999; Lücke, 1986). However, inactivation of *Enterobacteriaceae* during the whole shelf life of the product could only be prevented with HPP (Figure 2). Pressurisation (600 MPa, 5 min, 12ºC) of sliced fermented sausages induced an immediate reduction of *Enterobacteriaceae* counts (day 1). As other Gram-negative bacteria, *Enterobacteriaceae* has proved to be sensitive to HPP in cured meat products (Latorre-Moratalla, Bover-Cid, Aymerich, Marcos, Vidal-Carou & Garriga, 2007; López-Caballero, Carballo & Jiménez-Colmenero, 2002; Rubio, Martinez, Garcia-Cachan, Rovira & Jaime, 2007).

Besides, no effect of active packaging was observed against *Enterobacteriaceae* population ($p>0.05$).

No significant differences among batches were observed at the end of the shelf life. All treatments led to a decrease of *Enterobacteriaceae* population below the detection limit (10 cfu/g) from day 60 and until the end of storage.

Lactic acid bacteria behaviour

Initial lactic acid bacteria (LAB) counts in fermented sausages were 5.83 ± 0.33 log cfu/g. Figure 3 shows the decrease of LAB counts during the shelf life of all studied batches ($p<0.001$).

Non-pressurised batches (C NT and N NT) showed a 1 log unit reduction of LAB population throughout storage. On the other hand, pressurised batches (C HPP and N HPP) showed reductions of about 2.4 log units at the end of storage.

In the control batch (C HPP), the effect of HPP on LAB was not detected immediately after pressurisation (day 1). On the contrary, N HPP allowed an immediate reduction of 1 log cfu/g of LAB after HPP. C HPP showed a reduction of LAB population from day 30 of refrigerated storage (Figure 3) reaching the same levels of reduction than N HPP at the end of the shelf life. Although no immediate inactivation of LAB was detected after HPP in C HPP batch, sublethal injuries caused by HPP would end up in a reduction of the population in a hostile environment with reduced a_w such as the studied fermented sausages.

The behaviour of LAB after a HHP treatment has been observed to be dependent on the strain and the food matrix. In agreement with the present study, no recovery of LAB was found in pressurized dry-cured ham and beef loin during 120 days of storage at 4ºC

(Jofré, Aymerich, Grèbol & Garriga, 2009). While Garriga, Grèbol, Aymerich, Monfort & Hugas (2004) reported the ability of endogenous LAB present in cooked ham to recover after a 600 MPa treatment during the storage of the product at 4ºC. Inactivation of LAB by HPP could have a negative effect on food safety and quality if HPP was performed before ripening (Marcos et al 2005). However, pressure induced inactivation of LAB would have a minor impact on food safety and quality when HPP is applied as a post-processing technology, when fermented sausages are stable due to their a_w values.

Conclusions

Sliced fermented sausages with no added sodium salt obtained with the QDS® process proved to be products not able to support *L. monocytogenes* growth. However, the pathogen would be able to survive during the product shelf-life, if post-processing contamination occurred. Antimicrobial packaging with the inclusion of nisin as a natural antimicrobial could be considered as an effective method to improve the safety of sliced fermented sausages with no added sodium salt obtained with the QDS® process.

L. monocytogenes was able to survive to high pressure processing (600 MPa, 5 min, 12ºC) due to the conditions of the product under study of low water activity and presence of lactate in its formulation. Therefore, HPP could not be considerate an appropriate treatment to reduce L. monocytogenes in the type of fermented sausage under study.

These results reflect the impact of the food matrix on the effectiveness of post-processing technologies, highlighting the importance of validating novel technologies using food products with the exact formulation to be commercialised.

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535 **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
[1]Potassium lactate (g/kg,77.8% purity)	28.15

536 [1]Added amount.

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Table 2. pH and water activity values during the shelf life of dry-fermented sausages (QDS®) with no added sodium salt.

		time (days)		
	batch	1	15	90
pH	C NT	5.70±0.04[ab]	5.60±0.04[b]	5.58±0.01
	C HPP	5.72±0.03[ab]	5.69±0.02[ab]	5.63±0.03
	N NT	5.66±0.04[b]	5.64±0.03[b]	5.60±0.01
	N HPP	5.75±0.04[a]	5.75±0.05[a]	5.59±0.02
	p	<0.05	<0.01	NS
aw	C NT	0.879±0.006	0.880±0.010	0.883±0.004
	C HPP	0.879±0.002	0.876±0.001	0.884±0.002
	N NT	0.878±0.008	0.887±0.001	0.883±0.002
	N HPP	0.876±0.005	0.888±0.008	0.887±0.005
	p	NS	NS	NS

C: control, N: nisin, NT: non-treated, HPP: high pressure processing, a_w: water activity, NS: p>0.05. Results are means of six replicates. Different letters within a column indicate significant differences among treatments.

549 **Figure 1**. Inactivation of *L. monocytogenes* during the shelf life of sliced dry-fermented
550 sausages (QDS®) with no added sodium salt. $|\log_{10} N/N_0|$ gives the absolute value of the level
551 of inactivation, where N is the *L. monocytogenes* count at each time point, and N_0 is the
552 inoculated level (5×10^5 CFU/g). C: control, N: nisin, NT: non-treated, HPP: pressurised (600
553 MPa, 5 min, 12ºC). Values are means ± standard deviation of six replicates. Different letters
554 within a sampling day mean significant differences among batches ($p<0.0001$).

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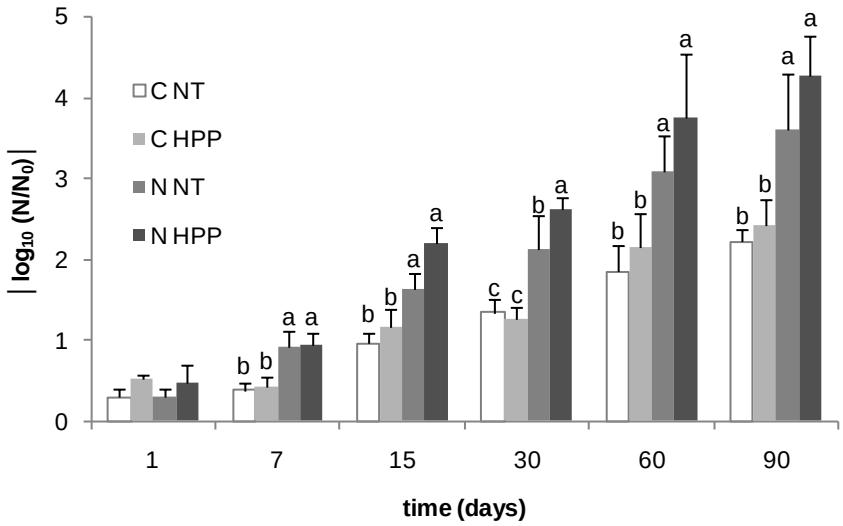

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Figure 2. Behaviour of *Enterobacteriaceae* during the shelf life of sliced dry-fermented sausages (QDS®) with no added sodium salt packed with C (control) and N (nisin) polyvinyl alcohol films as interleaves. NT: non-treated; HPP: high pressure processing (600 MPa, 5 min, 12 ºC). Values are means ± standard deviation of six replicates.

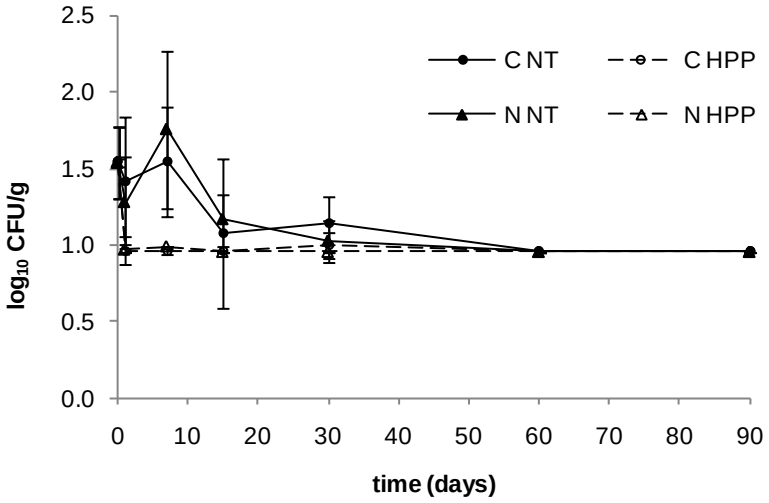

Figure 3. Behaviour of lactic acid bacteria during the shelf life of sliced dry-fermented sausages (QDS®) with no added sodium salt packed with C (control) and N (nisin) polyvinyl alcohol films as interleaves. NT: non-treated; HPP: high pressure processing (600 MPa, 5 min, 12 ºC). Values are means ± standard deviation of six replicates.