Assessment of High Hydrostatic Pressure and Starter Culture on the Quality Properties of Low-Acid Fermented Sausages

Begonya Marcos, Teresa Aymerich, M. Dolors Guardia, Margarita Garriga*

Institute for Food and Agricultural Research and Technology (IRTA), Meat Technology Centre, Granja Camps i Armet s/n, 17121 Monells, Spain

*Corresponding author.

Institute for Food and Agricultural Research and Technology (IRTA), Meat Technology Centre, Granja Camps i Armet s/n, 17121 Monells, Spain.

Tel. + 34 972 630 052
Fax. +34 972 630 373
E-mail address: margarita.garriga@irta.es
Abstract

The addition of starter culture and high pressure processing after ripening improved the microbial quality of low-acid fermented sausages (fuet and chorizo). The use of *Lactobacillus sakei* CTC6626 and *Staphylococcus xylosus* CTC6013 as starter culture significantly reduced *Enterobacteriaceae* and *Enterococcus* levels in the finished sausages. Moreover, the addition of starter culture produced sausages with similar quality properties to traditional low-acid fermented sausages. Slightly lower pH values and higher cohesiveness were obtained for both fuet and chorizo with starter culture. Sensory analysis showed no differences between lots of chorizo whereas starter fuet was more acid and gummy. High pressure induced an additional reduction of *Enterobacteriaceae* in non-starter sausages. An increase of texture properties was observed after pressurisation. No other differences were observed between non-treated and pressurized sausages.

*Keywords*: low-acid fermented sausages, traditional sausages, starter culture, high pressure processing
1. Introduction

The consumption of low-acid fermented sausages, with a limited acid taste, is common among Mediterranean countries. Sausages are dried at low temperatures (≤10-12°C) to avoid a rapid and intense fermentation, achieving final pH values of over 5.3 (Sanz, Vila, Toldrá, & Flores, 1998; Aymerich, Martín, Garriga, & Hugas, 2003). Many traditional slightly fermented sausages are produced by spontaneous meat fermentation, which cannot always guarantee the product to be safe and stable.

The addition of competitive starter cultures to lead the fermentation process is an effective method of inhibiting and/or controlling the growth of spoilage organisms and food-borne pathogens, preventing the formation of undesirable end-products (Rödel, Stiebing, & Kröckel, 1993; Lücke, 1998; Garriga et al., 2005). In Europe, starter cultures are made-up of a balance between the two main groups of bacteria that are responsible for meat fermentation: lactic acid bacteria (*Lactobacillus*) and Gram-positive catalase-positive cocci (*Staphylococcus*) (Hugas & Monfort, 1997; Talon, Leroy-Sétrin, & Fadda, 2002). Lactic acid bacteria (LAB) inhibit spoilage and pathogen development mainly as a result of competitive growth and acidification of the product. Acidification promotes the formation of colour and the cohesion of sausages (Lücke, 1998, 2000; Bacus, 1986). The addition of Gram-positive catalase-positive cocci (GCC+) to dry sausage manufacturing improves their sensorial properties (Nychas & Arkoudelos, 1990). GCC+ contribute to the development of dry sausage flavour by influencing the composition of volatile compounds in the products. In particular, they modulate the level and nature of volatiles originated from lipid oxidation (Berdagué, Monteil, Montel, & Talon, 1993; Montel, Reitz, Talon, Berdagué, & Rousset-Akrim, 1996; Talon, Walter, & Montel, 2000). GCC+ also ensure colour development by nitrate reductase activity (Lücke & Hechelmann, 1987).

High pressure processing (HPP) is a preservation method that kills and/or sub-lethally injures microorganisms mainly owing to membrane damage (Kalchayanand, Sikes, Dunne, & Ray, 1998). The pressure stability of vitamins and low molecular weight molecules, mainly responsible for odour and flavour (Smelt, 1998) makes this technology an interesting non-thermal alternative method to inactivate vegetative bacterial cells in RTE-foods. It is worth noting that besides destroying microorganisms there are further influences of pressure on food products to be expected, such as protein denaturation or modification, enzyme activation or inactivation, changes in enzyme-substrate interactions, changes in the properties of polymer carbohydrates and fats (Butz & Tauscher, 2002) that could affect the final quality of fermented sausages.
In a previous work (Garriga et al., 2005) the addition of starter culture consisting of LAB and GCC+ led to an improvement in safety and hygiene of low-acid fermented sausages inoculated with pathogens. However, pressurization proved to be necessary to assure absence of Salmonella spp. in the finished product.

The aim of this study was to evaluate the impact of the addition of a starter culture (consisting of selected strains of *L. sakei* and *S. xylosus*) and HPP on the microbial, chemical, physical and sensory properties of two types of traditional Spanish low-acid fermented sausages (fuet and chorizo).

**2. Materials and methods**

**2.1. Low-acid fermented sausage manufacture**

Two types of low-acid fermented sausages, fuet (F) and chorizo (C), were manufactured. Both products were made with pork bellies and shoulders. The meat was minced at −1°C in a meat cutter (Tecmaq, Barcelona, Spain) with an adjustable plate set at a hole diameter of 6 mm. A shoulder:belly proportion of 50:50 was mixed with common additives in a mixer machine (model 35P, Tecnotrip S.A., Terrassa, Spain). The formulation of fuet was as follows (g per kg): sodium chloride 20, black pepper 2.5, potassium nitrate 0.1, sodium nitrite 0.1, dextrose 1, and sodium ascorbate 0.5. The formulation of chorizo was (g per kg): sodium chloride 20, cayenne pepper 15, paprika 15, dextrose 1, and dehydrated garlic 3. Two different lots of each product were manufactured: lot 1, non-starter, and lot 2, inoculated with a starter culture consisting of *L. sakei* CTC6626 and *S. xylosus* CTC6013. *L. sakei* and *S. xylosus* were inoculated to achieve $4 \times 10^5$ and $4 \times 10^6$ CFU/g, respectively per sausage for each species. The mixture was stuffed in collagen casings (Colex 32 mm, Fibran S.A., Girona, Spain), each lot consisted of 28 sausages (350 g each sausage). Sausages were ripened at 12°C and 80% of relative humidity for 28 days.

**2.2. High pressure processing**

After 28 days of ripening, half of sausages were no-treated (HPP-) and half were subjected to a high pressure treatment (HPP+). After vacuum packaging in polyamide-polyethylene bags (Sacoliva, Sabadell, Spain), the sausages were pressurized at 400 MPa for 10 minutes at 17°C. HPP was carried out in an industrial hydrostatic pressurization unit (Alstom, France) with a chamber of 320 l volume and 280 mm diameter. The pressurization fluid was water, the come up time was 17.5 min, the pressure release time was 1.5 min, and the adiabatic heat generated was 5°C.
2.3. Sampling procedure

During the ripening process, at selected times (0, 6 days), three individual sausages from each product (F, C) and lot (1, 2) were sampled for microbial counts, pH and water activity ($a_w$) determination. At the end of ripening (28 days), and after high pressure treatment three sausages from each product (F, C), lot (1, 2), and pressure treatment (HPP-, HPP+) combinations were sampled for microbial counts, pH, $a_w$, TBARS, colour and texture determination. For sensory analysis two individual sausages of each treatment combination were sampled.

2.4. Microbiological analysis

Twenty grams of sausage were 10-fold diluted in sterile 0.1% peptone water (Difco Laboratories, Detroit, Mich., U.S.A.) and 0.85% NaCl (Merck, Darmstadt, Germany). The solution was homogenized for 1 min in a Masticator (IUL Instruments, Barcelona, Spain). After appropriate dilutions, the following determinations were carried out: LAB were enumerated by pour plating in MRS agar (Merck, Darmstadt, Germany) incubated anaerobically at 30°C for 72 h; GCC+ were enumerated by spread plating in mannitol salt agar, MSA (Difco Laboratories) incubated at 30°C for 48 h; Enterococcus were enumerated in poured kanamycin-esculin-azide agar (Oxoid, Basingstoke, Hampshire, England) incubated at 37°C for 24 h; Enterobacteriaceae were enumerated by pour plating in violet red bile glucose agar (Merck) at 30°C for 24 h.

2.5. Strain typing

Twenty colonies of LAB and GCC+ per lot were randomly selected from the MRS and MSA agar plates respectively, and used for implantation control of starter cultures in the finished products. Total DNA isolation was performed as previously reported (Aymerich et al., 2003). To achieve the lysis of GCC+ cells, lysostaphin (55 U/ml) was added to the lysis step. Strain typing was assessed by random amplification of polymorphic DNA (RAPD) PCR as previously reported (Martín, Garriga, Hugas, & Aymerich, 2005). Two random primers (Roche Molecular Biochemicals, Indianapolis, Ind.) were used for RAPD analysis, R5 (59-aacgcgcac) and M13R2 (59-ggaacagctatgaccatga). The banding profiles were visualized under UV light and digitalized by the Gelprinter photodocumentation equipment (TDI, Barcelona, Spain). Electrophoretic profiles obtained were normalized and analyzed by the software Fingerprinting II (Bio-Rad Laboratories, Hercules, Calif.).
2.6. pH, water activity, nitrate and nitrite measurements

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 sausage. Water activity ($a_w$) measurement was carried out using a Novasina Thermoconstanter TH-500 (Novasina, Switzerland) at 25 ºC.

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.

2.7. Measurement of lipid oxidation

The extent of lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS). The extraction method used was based on the procedure of Botsoglou, et al. (1994). After mincing, a sample of 2.5 g was homogenized with 20 ml of ultra pure water. Five ml of 25% trichloroacetic acid (Sigma-Aldrich, Saint Louis, MO, U.S.A.) were added to the homogenate. The solution was centrifuged at 4ºC for 15 min at 13,000 × g. After filtration, 3.5 ml of the extract were incubated with 1.5 ml of 0.6% aqueous 2-thiobarbituric acid (Sigma-Aldrich) for 30 min at 70ºC, and cooled with ice. The absorbance was measured at 532 nm using a UV-240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The concentration was calculated using a standard curve of malonaldehyde (0-2.5ng) (Sigma-Aldrich). Stock MDA solution (250µgMDA/ml) was obtained after hydrolysis of 1,1,3,3-tetraethoxypropane (TEP) in 10 ml of 0.1N HCl. The solution was immersed in a bath of boiling water for 5 min and quickly cooled. 1ml of hydrolyzed TEP was diluted to 250ml with ultrapure water. Results were expressed as micrograms of malonaldehyde (MDA) per gram of sausage.

2.8. Instrumental colour measurement

Instrumental colour measurements of sausages were performed using a Minolta Chromameter CR200 (Minolta, Japan). C illuminant and 2º standard observer were chosen. L* (lightness), a* (redness), and b* (yellowness) colour values were determined in the 1976 CIELAB system. The colorimeter was calibrated before each series of measurements using a white ceramic plate. The mean of six measurements was recorded for each sausage.
2.9. Texture Profile Analysis (TPA)

A MTS Texture Analyser (MTS Systems Corporation, MN, USA) was used to carry out a Texture Profile Analysis (TPA: Bourne, 1978) of the finished products. TPA is an imitative test that simulates 2 consecutive bites by way of two cycles of compression. The samples (1x1x1 cm) were compressed to 75 % of their original height using a crosshead speed of 1 mm/s. The following parameters were determined: hardness (Kg), cohesiveness (dimensionless), springiness (dimensionless), and chewiness (Kg). The mean of six measurements per sausage was recorded.

2.10. Sensory analysis

Six trained assessors (ASTM, 1981) undertook the sensory analysis on 1 cm thick slices. The generation and selection of the descriptors was carried out by open discussion in three sessions. Both products were checked for appearance (colour intensity), odour (overall intensity, rancid), acid taste, flavour (rancid, cured, cooked, off-flavours) and texture (hardness, gumminess). A non-structured 10-point scoring scale (Amerine, Pangborn, & Roessler, 1965) was used, where 0 means absence or very low intensity of the descriptor and 10 means very high intensity of the descriptor. Means of scores given by the assessors for each sausage were recorded. Evaluation was undertaken in four sessions (two sessions per product). A randomized complete block design (Steel & Torrie, 1983) was used in the sensory sessions, testing four sausages per session.

2.11. Statistical analysis

Data from each product (fuet and chorizo) were analysed separately using the GLM procedure from the SAS statistical package (SAS © System for Windows, Release 8.2, SAS Institute, Cary, NC, USA). The model for pH, a₅, and microbiological data recorded during ripening (0, 6, 28 days) included lot, time, and their interaction as fixed effects. The model for pH, a₅, microbiological, TBARS, instrumental colour, TPA, and sensory data recorded in the finished product included lot (1, 2), pressure treatment (HPP⁺, HPP⁻) and their interaction as fixed effects. Session was also added to the model as a fix effect for data from sensory analysis. Non significant interactions (p>0.05) were dropped from the model. Differences were assessed by the tukey test (p<0.05).

The percentage of implantation of a given inoculated strain was ascertained according to a sampling plan based on the binomial distribution (Peña Sánchez de Rivera, 1986). The implantation breakpoint,
defined as percentage of strains that showed the same RAPD profile as the added starter cultures, was set up at 83%.

3. Results

3.1. Water activity, pH, nitrate and nitrite content

During ripening the water activity ($a_w$) of sausages decreased ($p<0.05$) from an initial value of 0.98±0.01 in the raw product to values of 0.86-0.88 at day 28 of ripening. No other significant effect on $a_w$ was observed.

Interactions between lot and time were significant for pH values, results of the interaction are shown in figure 1. No decrease of pH ($p>0.05$) was observed during ripening of non-starter sausages (lot 1), showing final values of 5.80±0.03 and 5.71±0.01 in F1 and C1, respectively. Oppositely, sausages inoculated with starter culture (lot 2) showed a pronounced decrease ($p<0.05$) of pH values during the first days of ripening, reaching minimum values at day 6 of process (Fig. 1).

No changes of pH and $a_w$ values ($p>0.05$) were observed as a consequence of pressurization (400 MPa) of ripened sausages (Tables 1 and 2).

Fuet contained 100 ppm of nitrate and 100 ppm of nitrite as additives, whereas chorizo contained only 54 ppm of nitrate and 0.38 ppm of nitrite supplied by cayenne pepper and paprika.

3.2. Microbiological analysis

Interactions between lot and time were significant for microbiological data; results of the interaction are shown in figure 2. Initial LAB levels of $10^5$ CFU/g were observed in all lots, both non-starter and starter. Endogenous LAB (lot 1) grew gradually during the process, reaching values at the end of ripening of 7.25±0.10 and 7.78±0.13 log CFU/g in F1 and C1, respectively. LAB of starter sausages (lot 2), though, experienced a sharp increase in growth ($p<0.05$), reaching counts of 9.25±0.48 log CFU/g at day 6, values that were maintained until the end of ripening (Fig. 2a). LAB levels of lot 2 were, thus, significantly higher than those of lot 1 throughout the process. Initial levels of endogenous GCC+ were 3.51±0.18 and 6.59±0.19 log CFU/g in non-starter sausages (F1, C1), and starter sausages (F2, C2), respectively. No differences ($p>0.05$) were found in GCC+ counts between C1 and C2 at the end of ripening (6.87±0.40 log CFU/g), whereas significantly higher counts ($p<0.05$) were observed in F2 compared to F1 (7.87±0.24 and 6.61±0.20 log CFU/g, respectively). Implantation of
inoculated starter cultures was monitored by RAPD PCR. At the end of ripening 100% of GCC+ isolates presented identical fingerprints to *S. xylosus* CTC6013 in F2, whereas it represented 65% of isolates in C2. Among LAB isolates, 100% yielded the *L. sakei* CTC6626 genotype both in F2 and C2.

Figure 2b shows the evolution of *Enterobacteriaceae* population during ripening. In non-starter sausages *Enterobacteriaceae* counts increased in 2.6 log units during the first 6 days. These levels (5 log CFU/g) were maintained until the end of ripening in C1, whereas they were reduced to initial counts in F1. In starter sausages F2 showed no growth of *Enterobacteriaceae* during the first 6 days of ripening, while C2 showed an increase of 2.5 log units. All starter sausages attained *Enterobacteriaceae* counts under the detection limit (10 CFU/g) at the end of ripening. *Enterococcus* counts of non-starter sausages increased (p<0.05) during the process in 2.1 and 2.8 log units in F1 and C1, respectively. By contrast, enterococcal population of starter sausages decreased 1 logarithm (p<0.05) during ripening (Fig. 2c).

Interaction between lot and pressure treatment was significant, for GCC+, and *Enterobacteriaceae* data in both products, for LAB data in fuet, and for *Enterococcus* data in chorizo. For LAB counts pressurization only reduced the counts (p<0.05) of F1 (from 7.25±0.10 to 6.42±0.28 log CFU/g). On the other hand, after pressurization, GCC+ levels in F2 (7.87±0.24 log CFU/g) and C2 (7.44±0.39 log CFU/g) were significantly reduced in 0.83 and 0.92 log CFU/g, respectively. Regarding *Enterobacteriaceae* population, HPP reduced the counts (p<0.05) of F1 (1.98± 0.52 log CFU/g) and C1 (4.74±0.01 log CFU/g) in 1 and 3.8 logarithms, respectively, both reaching counts under the detection limit. For *Enterococcus* counts pressurisation only reduced the counts (p<0.05) of C1 (from 5.39±0.35 to 3.35±0.38 log CFU/g).

### 3.3. Lipid oxidation: TBARS

Interaction between lot and pressure treatment was not significant (p>0.05). Tables 1 and 2 show TBARS values of fuet and chorizo after 28 days of ripening. No significant effect (p>0.05) was observed as a consequence of either addition of starter culture or pressure treatment.

### 3.4. Instrumental colour analysis

Interaction between lot and pressure treatment was not significant (p>0.05). Colour parameters of low-acid fermented sausages at the end of processing are presented in Tables 1 and 2. No significant differences were observed among lots of fuet (Table 1). The addition of starter culture to chorizo (C2)
led to sausages with higher a* (redness) values than in the control lot, C1 (Table 2). None of the
colour parameters of fuet and chorizo were modified by pressurization at 400 MPa.

3.5. Texture Profile Analysis (TPA)

Interaction between lot and pressure treatment was not significant (p>0.05). The results of the TPA
applied to fuet and chorizo after 28 days of ripening are shown in Tables 1 and 2. Higher values of
cohesiveness (p<0.05) were observed in starter sausages (lot 2) than in non-starter ones (lot 1). F2
showed higher values of chewiness and springiness (p<0.05) than F1. Pressurization of sausages at
400 MPa increased (p<0.05) the cohesiveness, chewiness and springiness of both fuet and chorizo
(Tables 1 and 2).

3.6. Sensory analysis

Interaction between lot and pressure treatment was not significant (p>0.05). Results of the sensory
analysis of ripened fuet and chorizo are shown in Tables 3 and 4, respectively. The trained assessors
detected higher colour intensity and stronger cured flavour in C1 than in C2. Besides, starter fuet (F2)
were scored with higher acid taste and gummier texture than control fuet (F1). Pressurization induced
no changes (p>0.05) in the sensory properties of fuet, while a slight decrease (p<0.05) of colour
intensity was detected by the assessors in pressurized chorizo.
4. Discussion

Effect of starter culture

In the manufacture of low-acid fermented sausages, the selection of the appropriate strains used as starter cultures becomes essential to obtain products with the characteristic quality attributes of the traditional products.

The addition of starter culture, *L. sakei* CTC6626, which dominated over endogenous LAB, assured the pH drop in fuet and chorizo. Nevertheless, all sausages studied presented final pH values ≥ 5.3, which are common values for this type of low-acid fermented sausages (Sanz et al., 1998; Aymerich et al., 2003). Starter sausages led to a decrease of *Enterobacteriaceae* population below the detection limit. However, its growth during the first days of ripening could only be prevented in starter fuet, probably due to the highest content of nitrate and nitrite. Besides, the addition of the starter culture led to the reduction of *Enterococcus* levels during ripening of starter lots. The control of this population by starter cultures seemed to be more related to competitive exclusion than to pH, since enterococci are considered highly resistant to extreme pH values (Giraffa, 2002; Garriga et al., 2005). The control of *Enterobacteriaceae* and *Enterococcus* growth throughout the process is essential to prevent quality defects such as the formation of off-flavours and the production of biogenic amines (Maijala, Eerola, Lievonen, Hill, & Hirvi, 1995; Garriga et al., 1996), therefore the combination of hurdles proved to be necessary to improve the microbial quality of the product.

GCC+ influence the quality of the product by contributing to the oxidation of free fatty acids and colour formation during ripening. Oxidation is necessary to develop the desirable flavour of fermented sausages, but it is also one of the primary mechanisms of quality deterioration (Madsen & Bertelsen, 1995; Aguirrezábal, Mateo, Domínguez, & Zumalacárregui, 2000). No differences on TBARS values, used as a measure of lipid oxidation, were observed between lots coinciding with low values of rancidity observed in the sensory analysis. Love & Pearson (1974) related TBARS numbers greater than 1.0 to the detection of off-flavours, however no off-flavours were detected in our study in samples with TBARS values in a range of 1 to 2. No colour differences were found among lots of fuet, it can be deduced that both endogenous and inoculated GCC+ influenced colour formation in the same way, leading to end products with similar colour parameters. However, in chorizo lower a* values were observed in non-inoculated lot. Fernández-López, Pérez-Álvarez, Sayas-Barberá, & López-Santoveña (2002) suggested that a decrease of a* in chorizo could reflect an incipient oxidation of paprika.
The addition of starter culture affected textural characteristics of fermented sausages, it might be due mainly to their proteolytic activity and differences in pH values of sausages. Starter sausages, which were more acid than non-starter, showed higher cohesiveness, chewiness and springiness in fuet and higher values of cohesiveness in chorizo. Thus, the values of the textural properties increased as the pH values of sausages came near to the isoelectric point of meat proteins (5.3). Gimeno, Ansorena, Astiasarán, & Bello (2000) reported negative correlations of cohesiveness, chewiness and hardness with pH values in chorizo. In the sensory analysis, the assessors found starter fuet gummier than non-starter ones. The increased sensory gumminess could be related to the higher instrumental springiness detected by the TPA.

Effect of high pressure processing

High pressure processing (400 MPa) at the end of ripening reduced Enterobacteriaceae population of non-starter sausages. Besides, Enterococcus levels were reduced in non-starter chorizo, confirming the fact that sensitivity of enterococcal population to HPP presents a great variability influenced by the numbers and spices composition of each specific product (Martín et al., 2005).

No changes of colour parameters were observed after pressurization of fermented sausages. The ripening process leads to colour stabilization by turning myoglobin into nitrosylmyoglobin and nitrosylhaemochrome, which are not affected by pressure (Carlez et al., 1995; Cheftel & Culioli, 1997). Goutefongea, Rampon, Nicolas, & Dumont (1995) also reported no changes in a* and b* values after pressurization of cured meat at 600 MPa, while L* component was increased. However, a slight decrease of colour intensity in pressurized chorizo with respect to non-treated ones was detected in the sensory analysis. Anyway, it should be underlined that the difference detected by the trained panel (0.3 point difference), rarely could be detected by consumers. No alteration of TBARS values was detected in the present study, probably due to the fact that most lipid oxidation had already occurred during ripening. Andrés, Adamsen, Møller, Ruiz, & Skibsted (2005) reported a loss of oxidative stability of dry-cured ham pressurized at 400 MPa, during subsequent air-storage.

Pressurized sausages showed higher cohesiveness, chewiness and springiness than non-treated sausages. Yuste, Mor-Mur, Capellas, & Pla (1999) and Mor-Mur & Yuste (2003) also observed an increase of cohesiveness in sausages treated at 500 MPa at 65°C. No texture changes induced by HPP were detected by the assessor, suggesting that its effect would not be detectable by consumers.
5. Conclusions

Although starter cultures have proved to be important in improving hygiene and safety, the selection of optimum starter cultures that do not modify sensorial properties of traditional slightly fermented sausages is essential. The addition of starter culture (L. sakei CTC6626 and S. xylosus CTC6013) produced sausages with improved hygiene and similar quality properties to traditional low-acid fermented sausages.

High pressure processing (400 MPa, 17°C, 10 min) after ripening also improved hygiene of sausages, without damaging the quality of the product. Considering those results and the fact that this pressure treatment is effective in improving product safety (Marcos, Aymerich, & Garriga 2005; Garriga et al., 2005), the use of high pressure treatment can be recommended as a final step in the manufacturing process of low-acid fermented sausages with appropriate starter cultures.

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References


Figure captions

Figure 1. Evolution of pH during ripening of fuet (F) and chorizo (C), non-starter (1), and inoculated with starter culture (2). Values are the mean of triplicate sausages.

Figure 2. Evolution of lactic acid bacteria (a), Enterobacteriaceae (b) and Enterococcus (c) population during ripening of fuet (F) and chorizo (C), non-starter (1), and inoculated with starter culture (2). Values are the mean of triplicate sausages.
Table 1. pH, aw, TBARS colour and texture profile analysis (TPA) parameters of fuet measured at the end of ripening (day 28).

<table>
<thead>
<tr>
<th>Lot (n=6)</th>
<th>Treatment</th>
<th>Root MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>HPP- (n=6)</td>
</tr>
<tr>
<td>aw</td>
<td>0.858</td>
<td>0.878</td>
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<tr>
<td>pH</td>
<td>5.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.53&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>L&lt;sup&gt;*&lt;/sup&gt;</td>
<td>39.19</td>
<td>40.34</td>
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<tr>
<td>a&lt;sup&gt;*&lt;/sup&gt;</td>
<td>10.39</td>
<td>10.74</td>
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<tr>
<td>b&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.26</td>
<td>3.26</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.151&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.178&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Hardness (Kg)</td>
<td>12.12</td>
<td>11.80</td>
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<td>Chewiness (Kg)</td>
<td>0.449&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.628&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Springiness</td>
<td>0.231&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.291&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TBARS (µg MDA/Kg)</td>
<td>1.96</td>
<td>1.69</td>
</tr>
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</table>

<sup>a,b</sup> different letters indicate significant differences between lots.
<sup>x,y</sup> different letters indicate significant differences between treatments.
1: non-starter; 2: inoculated starter culture.
HPP-: non-treated; HPP+: pressurized.
Table 2. pH, aw, TBARS colour and texture profile analysis (TPA) parameters of chorizo measured at the end of ripening (day 28).

<table>
<thead>
<tr>
<th>Lot</th>
<th>Treatment</th>
<th>Root MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=6)</td>
<td>2 (n=6)</td>
<td>HPP- (n=6)</td>
</tr>
<tr>
<td>aw</td>
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<td>0.854</td>
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<td>pH</td>
<td>5.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.33&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>L*</td>
<td>36.55</td>
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<tr>
<td>a*</td>
<td>13.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>b*</td>
<td>9.84</td>
<td>10.90</td>
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<tr>
<td>Cohesiveness</td>
<td>0.184&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.197&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hardness (Kg)</td>
<td>12.34</td>
<td>13.08</td>
</tr>
<tr>
<td>Chewiness(Kg)</td>
<td>0.592</td>
<td>0.676</td>
</tr>
<tr>
<td>Springiness</td>
<td>0.254</td>
<td>0.261</td>
</tr>
<tr>
<td>TBARS (µg MDA/Kg)</td>
<td>1.21</td>
<td>1.02</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> different letters indicate significant differences between lots.
<sup>x,y</sup> different letters indicate significant differences between treatments.

1: non-starter; 2: inoculated starter culture.
HPP-: non-treated; HPP+: pressurized.
Table 3. Sensory properties of fuet evaluated at the end of ripening (day 28).

<table>
<thead>
<tr>
<th>Lot</th>
<th>Colour intensity</th>
<th>Odour intensity</th>
<th>Rancid odour</th>
<th>Acid taste</th>
<th>Cooked flavour</th>
<th>Rancid flavour</th>
<th>Off-flavours</th>
<th>Hardness</th>
<th>Gumminess</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=4)</td>
<td>6.2</td>
<td>5.0</td>
<td>0.9</td>
<td>0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3</td>
<td>1.1</td>
<td>0.2</td>
<td>3.9</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 (n=4)</td>
<td>5.9</td>
<td>5.1</td>
<td>1.6</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6</td>
<td>1.3</td>
<td>0.4</td>
<td>3.6</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HPP- (n=4)</td>
<td>6.1</td>
<td>5.2</td>
<td>1.3</td>
<td>1.9</td>
<td>0.1</td>
<td>0.9</td>
<td>0.2</td>
<td>3.7</td>
<td>1.8</td>
</tr>
<tr>
<td>HPP+ (n=4)</td>
<td>6.0</td>
<td>4.9</td>
<td>1.2</td>
<td>2.0</td>
<td>0.7</td>
<td>1.5</td>
<td>0.4</td>
<td>3.8</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Root MSE: 0.3573, 0.5665, 0.4071, 0.3451, 0.3723, 0.5809, 0.3140, 0.6382, 0.4439

<sup>a,b</sup> different letters indicate significant differences between lots.
<sup>x,y</sup> different letters indicate significant differences between treatments.
1: non-starter; 2: inoculated starter culture.
HPP-: non-treated; HPP+: pressurized.
**Table 4.** Sensory properties of chorizo evaluated at the end of ripening (day 28).

<table>
<thead>
<tr>
<th></th>
<th>Lot 1 (n=4)</th>
<th>Lot 2 (n=4)</th>
<th>HPP- (n=4)</th>
<th>HPP+ (n=4)</th>
<th>Root MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour intensity</td>
<td>8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;x&lt;/sup&gt;</td>
<td>7.6&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.1408</td>
</tr>
<tr>
<td>Odour intensity</td>
<td>6.0</td>
<td>5.9</td>
<td>5.6</td>
<td>6.3</td>
<td>0.3112</td>
</tr>
<tr>
<td>Rancid odour</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2813</td>
</tr>
<tr>
<td>Acid taste</td>
<td>1.2</td>
<td>2.5</td>
<td>1.7</td>
<td>2.0</td>
<td>0.7681</td>
</tr>
<tr>
<td>Cured flavour</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1</td>
<td>2.4</td>
<td>0.1876</td>
</tr>
<tr>
<td>Rancid flavour</td>
<td>0.2</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4102</td>
</tr>
<tr>
<td>Off-flavours</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2633</td>
</tr>
<tr>
<td>Hardness</td>
<td>4.4</td>
<td>3.9</td>
<td>4.1</td>
<td>4.1</td>
<td>0.8071</td>
</tr>
<tr>
<td>Gumminess</td>
<td>1.5</td>
<td>2.0</td>
<td>1.6</td>
<td>1.8</td>
<td>0.5160</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> different letters indicate significant differences between lots.
<sup>x,y</sup> different letters indicate significant differences between treatments.

1: non-starter; 2: inoculated starter culture.
HPP-: non-treated; HPP+: pressurized.
**Figure 1.**

![Graph showing pH over time for different conditions: F1, F2, C1, C2.](image)

**Figure 2.**

a)  

![Graph showing log CFU/g over time for different conditions: F1, F2, C1, C2.](image)