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 (≤10-12ºC) 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 –1ºC 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 $4x10^5$ and $4x10^6$ 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 12ºC 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 17ºC. 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 5ºC.

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-aacgcgcaac) and M13R2 (59-ggaaacagctatgaccatga). 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.).

138 *2.6. pH, water activity, nitrate and nitrite measurements*

139 The pH was measured directly in the samples using a Crison penetration 52-32 electrode connected
140 to a Crison Basic 20 pH-meter (Crison Instruments S.A., Alella, Spain). The mean of three
141 measurements was recorded for each sausage. Water activity (a_w) measurement was carried out
142 using a Novasina Thermoconstanter TH-500 (Novasina, Switzerland) at 25 ºC.

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

146

147 *2.7. Measurement of lipid oxidation*

148 The extent of lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS). The
149 extraction method used was based on the procedure of Botsoglow, et al. (1994). After mincing, a
150 sample of 2.5 g was homogenized with 20 ml of ultra pure water. Five ml of 25% trichloroacetic acid
151 (Sigma-Aldrich, Saint Louis, MO, U.S.A.) were added to the homogenate. The solution was
152 centrifuged at 4ºC for 15 min at 13,000 × *g*. After filtration, 3.5 ml of the extract were incubated with
153 1.5 ml of 0.6% aqueous 2-thiobarbituric acid (Sigma-Aldrich) for 30 min at 70ºC, and cooled with ice.
154 The absorbance was measured at 532 nm using a UV-240 spectrophotometer (Shimadzu
155 Corporation, Kyoto, Japan). The concentration was calculated using a standard curve of
156 malonaldehyde (0-2,5ng) (Sigma-Aldrich). Stock MDA solution (250μgMDA/ml) was obtained after
157 hydrolysis of 1,1,3,3-tetraethoxypropane (TEP) in 10 ml of 0.1N HCl. The solution was immersed in a
158 bath of boiling water for 5 min and quickly cooled. 1ml of hydrolyzed TEP was diluted to 250ml with
159 ultrapure water. Results were expressed as micrograms of malonaldehyde (MDA) per gram of
160 sausage.

161

162 *2.8. Instrumental colour measurement*

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

168

169 *2.9. Texture Profile Analysis (TPA)*

170 A MTS Texture Analyser (MTS Systems Corporation, MN, USA) was used to carry out a Texture
171 Profile Analysis (TPA: Bourne, 1978) of the finished products. TPA is an imitative test that simulates 2
172 consecutive bites by way of two cycles of compression. The samples (1x1x1 cm) were compressed to
173 75 % of their original height using a crosshead speed of 1 mm/s. The following parameters were
174 determined: hardness (Kg), cohesiveness (dimensionless), springiness (dimensionless), and
175 chewiness (Kg). The mean of six measurements per sausage was recorded.

176

177 *2.10. Sensory analysis*

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

187

188 *2.11. Statistical analysis*

189 Data from each product (fuet and chorizo) were analysed separately using the GLM procedure from
190 the SAS statistical package (SAS © System for Windows, Release 8.2, SAS Institute, Cary, NC, USA).

191 The model for pH, a_w, and microbiological data recorded during ripening (0, 6, 28 days) included lot,
192 time, and their interaction as fixed effects. The model for pH, a_w, microbiological, TBARS, instrumental
193 colour, TPA, and sensory data recorded in the finished product included lot (1, 2), pressure treatment
194 (HPP-, HPP+) and their interaction as fixed effects. Session was also added to the model as a fix
195 effect for data from sensory analysis. Non significant interactions ($p>0.05$) were dropped from the
196 model. Differences were assessed by the tukey test ($p<0.05$).

197 The percentage of implantation of a given inoculated strain was ascertained according to a sampling
198 plan based on the binomial distribution (Peña Sánchez de Rivera, 1986). The implantation breakpoint,

199 defined as percentage of strains that showed the same RAPD profile as the added starter cultures,
200 was set up at 83%.
201
202 **3. Results**
203
204 *3.1. Water activity, pH, nitrate and nitrite content*
205 During ripening the water activity (a_w) of sausages decreased ($p<0.05$) from an initial value of
206 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
207 a_w was observed.
208 Interactions between lot and time were significant for pH values, results of the interaction are shown
209 in figure 1. No decrease of pH ($p>0.05$) was observed during ripening of non-starter sausages (lot 1),
210 showing final values of 5.80±0.03 and 5.71±0.01 in F1 and C1, respectively. Oppositely, sausages
211 inoculated with starter culture (lot 2) showed a pronounced decrease ($p<0.05$) of pH values during the
212 first days of ripening, reaching minimum values at day 6 of process (Fig. 1).
213 No changes of pH and a_w values ($p>0.05$) were observed as a consequence of pressurization (400
214 MPa) of ripened sausages (Tables 1 and 2).
215 Fuet contained 100 ppm of nitrate and 100 ppm of nitrite as additives, whereas chorizo contained
216 only 54 ppm of nitrate and 0.38 ppm of nitrite supplied by cayenne pepper and paprika.
217
218 *3.2. Microbiological analysis*
219 Interactions between lot and time were significant for microbiological data; results of the interaction
220 are shown in figure 2. Initial LAB levels of 10^5 CFU/g were observed in all lots, both non-starter and
221 starter. Endogenous LAB (lot 1) grew gradually during the process, reaching values at the end of
222 ripening of 7.25±0.10 and 7.78±0.13 log CFU/g in F1 and C1, respectively. LAB of starter sausages
223 (lot 2), though, experienced a sharp increase in growth ($p<0.05$), reaching counts of 9.25±0.48 log
224 CFU/g at day 6, values that were maintained until the end of ripening (Fig. 2a). LAB levels of lot 2
225 were, thus, significantly higher than those of lot 1 throughout the process. Initial levels of endogenous
226 GCC+ were 3.51±0.18 and 6.59±0.19 log CFU/g in non-starter sausages (F1, C1), and starter
227 sausages (F2, C2), respectively. No differences ($p>0.05$) were found in GCC+ counts between C1 and
228 C2 at the end of ripening (6.87±0.40 log CFU/g), whereas significantly higher counts ($p<0.05$) were
229 observed in F2 compared to F1 (7.87±0.24 and 6.61±0.20 log CFU/g, respectively). Implantation of

230 inoculated starter cultures was monitored by RAPD PCR. At the end of ripening 100% of GCC+
231 isolates presented identical fingerprints to *S. xylosus* CTC6013 in F2, whereas it represented 65% of
232 isolates in C2. Among LAB isolates, 100% yielded the *L. sakei* CTC6626 genotype both in F2 and C2.
233 Figure 2b shows the evolution of *Enterobacteriaceae* population during ripening. In non-starter
234 sausages *Enterobacteriaceae* counts increased in 2.6 log units during the first 6 days. These levels (5
235 log CFU/g) were maintained until the end of ripening in C1, whereas they were reduced to initial
236 counts in F1. In starter sausages F2 showed no growth of *Enterobacteriaceae* during the first 6 days
237 of ripening, while C2 showed an increase of 2.5 log units. All starter sausages attained
238 *Enterobacteriaceae* counts under the detection limit (10 CFU/g) at the end of ripening. *Enterococcus*
239 counts of non-starter sausages increased ($p<0.05$) during the process in 2.1 and 2.8 log units in F1
240 and C1, respectively. By contrast, enterococcal population of starter sausages decreased 1 logarithm
241 ($p<0.05$) during ripening (Fig. 2c).
242 Interaction between lot and pressure treatment was significant, for GCC+, and *Enterobacteriaceae*
243 data in both products, for LAB data in fuet, and for *Enterococcus* data in chorizo. For LAB counts
244 pressurization only reduced the counts ($p<0.05$) of F1 (from 7.25±0.10 to 6.42±0.28 log CFU/g). On
245 the other hand, after pressurization, GCC+ levels in F2 (7.87±0.24 log CFU/g) and C2 (7.44±0.39 log
246 CFU/g) were significantly reduced in 0.83 and 0.92 log CFU/g, respectively. Regarding
247 *Enterobacteriaceae* population, HPP reduced the counts ($p<0.05$) of F1 (1.98± 0.52 log CFU/g) and
248 C1 (4.74±0.01 log CFU/g) in 1 and 3.8 logarithms, respectively, both reaching counts under the
249 detection limit. For *Enterococcus* counts pressurisation only reduced the counts ($p<0.05$) of C1 (from
250 5.39±0.35 to 3.35±0.38 log CFU/g).
251
252 *3.3. Lipid oxidation: TBARS*
253 Interaction between lot and pressure treatment was not significant ($p>0.05$). Tables 1 and 2 show
254 TBARS values of fuet and chorizo after 28 days of ripening. No significant effect ($p>0.05$) was
255 observed as a consequence of either addition of starter culture or pressure treatment.
256
257 *3.4. Instrumental colour analysis*
258 Interaction between lot and pressure treatment was not significant ($p>0.05$). Colour parameters of low-
259 acid fermented sausages at the end of processing are presented in Tables 1 and 2. No significant
260 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.

311 The addition of starter culture affected textural characteristics of fermented sausages, it might be
312 due mainly to their proteolytic activity and differences in pH values of sausages. Starter sausages,
313 which were more acid than non-starter, showed higher cohesiveness, chewiness and springiness in
314 fuet and higher values of cohesiveness in chorizo. Thus, the values of the textural properties
315 increased as the pH values of sausages came near to the isoelectric point of meat proteins (5.3).
316 Gimeno, Ansorena, Astiasarán, & Bello (2000) reported negative correlations of cohesiveness,
317 chewiness and hardness with pH values in chorizo. In the sensory analysis, the assessors found
318 starter fuet gummier than non-starter ones. The increased sensory gumminess could be related to the
319 higher instrumental springiness detected by the TPA.
320
321 *Effect of high pressure processing*
322 High pressure processing (400 MPa) at the end of ripening reduced *Enterobacteriaceae* population
323 of non-starter sausages. Besides, *Enterococcus* levels were reduced in non-starter chorizo, confirming
324 the fact that sensitivity of enterococcal population to HPP presents a great variability influenced by the
325 numbers and spices composition of each specific product (Martín et al., 2005).
326 No changes of colour parameters were observed after pressurization of fermented sausages. The
327 ripening process leads to colour stabilitzation by turning myoglobin into nitrosylmyoglobin and nitrosyl-
328 haemochrome, which are not affected by pressure (Carlez et al., 1995; Cheftel & Culioli, 1997).
329 Goutefongea, Rampon, Nicolas, & Dumont (1995) also reported no changes in a* and b* values after
330 pressurization of cured meat at 600 MPa, while L* component was increased. However, a slight
331 decrease of colour intensity in pressurized chorizo with respect to non-treated ones was detected in
332 the sensory analysis. Anyway, it should be underlined that the difference detected by the trained panel
333 (0.3 point difference), rarely could be detected by consumers. No alteration of TBARS values was
334 detected in the present study, probably due to the fact that most lipid oxidation had already occurred
335 during ripening. Andrés, Adamsen, Moller, Ruiz, & Skibsted (2005) reported a loss of oxidative stability
336 of dry-cured ham pressurized at 400 MPa, during subsequent air-storage.
337 Pressurized sausages showed higher cohesiveness, chewiness and springiness than non-treated
338 sausages. Yuste, Mor-Mur, Capellas, & Pla (1999) and Mor-Mur & Yuste (2003) also observed an
339 increase of cohesiveness in sausages treated at 500 MPa at 65ºC. No texture changes induced by
340 HPP were detected by the assessors, suggesting that its effect would not be detectable by
341 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, 17ºC, 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.

Acknowledgements

This work was supported by the National Institute for Food and Agricultural Research (INIA) projects RTA 01-084 and CPE 03-012-C3. B. Marcos is recipient of a pre-doctoral grant awarded by INIA. We thank L. Guerrero for his technical support in the sensory analysis.

References

Aguirrezábal, M. M., Mateo, J., Domínguez, M. C., & Zumalacárregui, J. M. (2000). The effect of paprika, garlic and salt on rancidity in dry sausages. *Meat Science, 54*(1), 77-81.

American Society for Testing and Materials. (1981). *Guidelines for the selection and training of sensory panel members. STP 758*. Philadelphia: ASTM.

Amerine, M., Pangborn, R., & Roessler, E. (1965). *Principles of sensory evaluation of food*. New York: Academic Press.

Andrés, A.I., Adamsen, C.E., Moller, J.K.S., Ruiz, J., Skibsted, L.H. (2005). High-pressure treatment of dry-cured Iberian ham. Effect on colour and oxidative stability during chill storage packed in modified atmosphere. *European Food Research Technology, 222*(5-6), 486-491.

Aymerich, M. T., Martín, B., Garriga, M., & Hugas, M. (2003). Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low-acid sausages*. Applied and Environmental Microbiology*, *69*(8), 4583-4594.

Bacus, J. N. (1986). Fermented meat and poultry products. In A. M. D. Pearson, T.R. (Ed.), Advances in *Meat and Poultry Microbiology* (pp. 123-164). London: Macmillan.

Berdagué, J. L., Monteil, P., Montel, M. C., & Talon, R. (1993). Effect of Starter Cultures on the Formation of flavour Compounds in Dry Sausage. *Meat Science*, *35*(3), 275-287.

376 Botsoglow, N. A., Fletuoris, D. J., Papageorgiu, G. E., Vassilopoulos, V. N., Mantis, A. J., &
377 Trakatellis, A. G. (1994). Rapid, sensitive and specific thiobarbituric acid method for measuring
378 lipid peroxidation in animal tissue, food and feedstuff samples. *Journal of Agricultural and Food*
379 *Chemistry*, *42*, 1931-1937.
380 Bourne, M. C. (1978). Texture profile analysis. *Food Technology*, *32*, 62-66, 72.
381 Butz, P., & Tauscher, B. (2002). Emerging technologies: chemical aspects. *Food Research*
382 *International*, *35*, 279-284.
383 Carlez, A., Veciana-Nogués, M. T., & Cheftel, J. C. (1995). Changes in colour and myoglobin of
384 minced meat due to high pressure processing. *Lebensmittel-Wissenschaft und-Technologie*, *28*,
385 528-538.
386 Cheftel, J. C., & Culioli, J. (1997). Effects of high pressure on meat: a review. *Meat Science*, *46*(3),
387 211-236.
388 Fernández-López, J., Pérez-Álvarez, J. A., Sayas-Barberá, M. E., & López-Santoveña, F. (2002).
389 Effect of paprika (Capsicum anuum) on color of Spanish-type sausages during the resting stage.
390 *Journal of Food Science*, *67*(6), 2410-2414.
391 Garriga, M., Hugas, M., Gou, P., Aymerich, M. T., Arnau, & J., Monfort, J. M. (1996). Technological
392 and sensorial evaluation of *Lactobacillus* strains as starter cultures in fermented sausages.
393 *International Journal of Food Microbiology*, *32*, 173-183.
394 Garriga, M., Marcos, B., Martín, B., Veciana-Nogués, M. T., Bover-Cid, S., Hugas, M., & Aymerich, M.
395 T. (2005). Starter cultures and high pressure processing to improve the hygiene and safety of
396 slightly fermented sausages. *Journal of Food Protection*, *68*(11), 2341-2348.
397 Gimeno, O., Ansorena, D., Astiasarán, I., & Bello, J. (2000). Characterization of chorizo de Pamplona:
398 instrumental measurements of colour and texture. *Food Chemistry*, *69*(2), 195-200.
399 Giraffa, G. (2002). Enterococci from foods. *FEMS Microbiology Reviews*, 26, 163-171.
400 Goutefongea, R., Rampon, V., Nicolas, J., & Dumont, J. P. (1995). Meat color changes under high
401 pressure treatment. *In Proceedings 41th International Congress of Meat Science and Technology*,
402 San Antonio, Texas.
403 Hugas, M., & Monfort, J. M. (1997). Bacterial starter cultures of food fermentation. Food Chemistry,
404 *59*(4), 547-554.
405 Kalchayanand, N., Sikes, A., Dunne, C. P., & Ray, B. (1998). Factors influencing death and injury of
406 foodborne pahogens by hydrostatic pressure-pasteurization. *Food Microbiology*, *15*(2), 207-214.
407 Love, J. D., & Pearson, A. M. (1974). Metmyoglobin and nonheme iron as prooxidants in cooked meat.
408 *Journal Agricultural and Food Chemistry*, *22*, 1032.
409 Lücke, F. K., & Hechelmann, H. (1987). Starter cultures for dry sausages and raw ham.
410 *Fleischwirtschaft*, *67*, 307-314.
411 Lücke, F. K. (1998). Fermented sausages. In B. J. B. Wood (Ed.), *Microbiology of Fermented Foods*
412 (vol. 2, pp. 441-483). London: Blackie Academic & Professional.
413 Lücke, F. K. (2000). Utilization of microbes to process and preserve meat. *Meat Science*, *56*(2), 105-
414 115.
415 Madsen, H. L., & Bertelsen, G. (1995). Species as antioxidants. *Trends in Food Science and*
416 *Technology*, *6*, 271-277.

Maijala, R., Eerola, S., Lievonen, S., Hill, P., & Hirvi, T. (1995). Formation of biogenic amines during fermentation of dry sausages as affected by starter culture an thawing time of raw materials. *Journal of Food Science*, *60*(6), 1187-1190.

Marcos, B., Aymerich, T., & Garriga, M. (2005). Evaluation of high pressure processing as an additional hurdle to control *Listeria monocytogenes* and *Salmonella enterica* in low-acid fermented sausages. *Journal of Food Science*, *70*(7), 339-344.

Martín, B., Garriga, M., Hugas, M., & Aymerich, T. (2005). Genetic diversity and safety aspects of enterococci from slightly fermented sausages. *Journal of Applied Microbiology*, *98*(5), 1177-1190.

Montel, M. C., Reitz, J., Talon, R., Berdagué, J. L., & Rousset-Akrim, S. (1996). Biochemical activities of Micrococcaceae and their effects on the aromatic profiles and odours of a dry sausage model. *Food Microbiology*, *13*(6), 489-499.

Mor-Mur, M., & Yuste, J. (2003). High pressure processing applied to cooked sausage manufacture: physical properties and sensory analysis. *Meat Science*, *65*(3), 1187-1191.

Nychas, G. J. E., & Arkoudelos, J. S. (1990). Staphylococci: their role in fermented sausages. *Journal of Applied Bacteriology Symposium Supplement*, 167S-188S.

Peña Sánchez de Rivera, D. (1986). Estadística. Modelos y métodos, 1. Fundamentos. Alianza Editorial, Madrid.

Rödel, W., Stiebing, A., & Kröckel, L. (1993). Ripening parameters for traditional dry sausages with a mould covering. *Fleischwirtschaft*, *73*, 848-853.

Sanz, Y., Vila, R., Toldrá, F., & Flores, J. (1998). Effect of nitrate and nitrite curing salts on microbial changes and sensory quality of non-fermented sausages. *International Journal of Food Microbiology, 42*(3), 213-217.

Smelt, J. P. P. M. (1998). Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology*, *9*, 152-158.

Sorensen, B. B. (1997). Lipolysis of pork fat by the meat sarter culture *Staphylococcus xylosus* at various environmental conditions. *Food Microbiology*, *14*(2), 153-160.

Steel, R. G. D., & Torrie, J. H. (1983). Principles and procedures of statistics. New York: Mc Graw-Hill.

Talon, R., Walter, D., & Montel, M. C. (2000). Growth and effect of staphylococci and lactic acid bacteria on unsaturated free fatty acids. *Meat Science*, *54*(1), 41-47.

Talon, R., Leroy-Sétrin, S., & Fadda, S. (2002). Bacterial starters involved in the quality of fermented meat products. In F. Toldrá (Ed.), *Research advances in the quality of meat and meat products* (pp. 175-191). Burjassot, Spain: Research Signpost.

Yuste, J., Mor-Mur, M., Capellas, M., & Pla, R. (1999). Pressure- vs. heat-induced bacterial stress in cooked poultry sausages: a preliminary study. *Letters in Applied Microbiology*, *29*, 233-237.

452 **Figure captions**

453

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

456

457 **Figure 2.** Evolution of lactic acid bacteria (a), *Enterobacteriaceae* (b) and *Enterococcus* (c) population
458 during ripening of fuet (F) and chorizo (C), non-starter (1), and inoculated with starter culture (2).
459 Values are the mean of triplicate sausages.

460

461

462 **Table 1.** pH, a_w, TBARS colour and texture profile analysis (TPA) parameters of fuet measured at the
463 end of ripening (day 28).

464

465
466

	Lot		Treatment		Root MSE
	1 (n=6)	2 (n=6)	HPP- (n=6)	HPP+ (n=6)	
aw	0.858	0.878	0.862	0.874	0.0167
pH	5.79 [a]	5.53 [b]	5.67	5.64	0.0431
L*	39.19	40.34	39.33	40.21	2.6321
a*	10.39	10.74	10.59	10.54	1.5373
b*	3.26	3.26	3.34	3.17	0.6400
Cohesiveness	0.151 [b]	0.178 [a]	0.152 [y]	0.177 [x]	0.0102
Hardness (Kg)	12.12	11.80	11.20	12.71	2.8059
Chewiness (Kg)	0.449 [b]	0.628 [a]	0.395 [y]	0.681 [x]	0.1218
Springiness	0.231 [b]	0.291 [a]	0.224 [y]	0.298 [x]	0.0198
TBARS (µg MDA/Kg)	1.96	1.69	0.92	1.77	0.6856

467
468 [a,b] different letters indicate significant differences between lots.
469 [x,y] different letters indicate significant differences between treatments.
470 1: non-starter; 2: inoculated starter culture.
471 HPP-: non-treated; HPP+: pressurized.
472
473

474 **Table 2.** pH, a_w, TBARS colour and texture profile analysis (TPA) parameters of chorizo measured at
475 the end of ripening (day 28).
476
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478
479

	Lot		Treatment		Root MSE
	1 (n=6)	2 (n=6)	HPP- (n=6)	HPP+ (n=6)	
aw	0.859	0.854	0.856	0.857	0.0129
pH	5.73 [a]	5.33 [b]	5.50	5.56	0.0271
L*	36.55	37.21	36.53	37.23	1.3944
a*	13.74 [b]	14.83 [a]	14.15	14.41	0.5767
b*	9.84	10.90	10.39	10.35	0.8613
Cohesiveness	0.184 [b]	0.197 [a]	0.182 [y]	0.199 [x]	0.0086
Hardness (Kg)	12.34	13.08	12.06	13.35	1.6707
Chewiness(Kg)	0.592	0.676	0.589 [y]	0.739 [x]	0.0958
Springiness	0.254	0.261	0.237 [y]	0.278 [x]	0.0161
TBARS (µg MDA/Kg)	1.21	1.02	0.46	0.80	0.2616

480
481 [a,b,]different letters indicate significant differences between lots.
482 [x,y] different letters indicate significant differences between treatments.
483 1: non-starter; 2: inoculated starter culture.
484 HPP-: non-treated; HPP+: pressurized.
485

Table 3. Sensory properties of fuet evaluated at the end of ripening (day 28).

	Lot		Treatment		Root MSE
	1 (n=4)	2 (n=4)	HPP- (n=4)	HPP+ (n=4)	
Colour intensity	6.2	5.9	6.1	6.0	0.3573
Odour intensity	5.0	5.1	5.2	4.9	0.5665
Rancid odour	0.9	1.6	1.3	1.2	0.4071
Acid taste	0.9 [b]	3.0 [a]	1.9	2.0	0.3451
Cooked flavour	0.3	0.6	0.1	0.7	0.3723
Rancid flavour	1.1	1.3	0.9	1.5	0.5809
Off-flavours	0.2	0.4	0.2	0.4	0.3140
Hardness	3.9	3.6	3.7	3.8	0.6382
Gumminess	1.3 [b]	2.3 [a]	1.8	1.9	0.4439

[a,b] different letters indicate significant differences between lots.
[x,y] 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).

	Lot		Treatment		Root MSE
	1 (n=4)	2 (n=4)	HPP- (n=4)	HPP+ (n=4)	
Colour intensity	8.1 [a]	7.4 [b]	7.9 [x]	7.6 [y]	0.1408
Odour intensity	6.0	5.9	5.6	6.3	0.3112
Rancid odour	0.4	0.2	0.3	0.3	0.2813
Acid taste	1.2	2.5	1.7	2.0	0.7681
Cured flavour	2.6 [a]	1.9 [b]	2.1	2.4	0.1876
Rancid flavour	0.2	0.5	0.4	0.4	0.4102
Off-flavours	0.3	0.2	0.2	0.3	0.2633
Hardness	4.4	3.9	4.1	4.1	0.8071
Gumminess	1.5	2.0	1.6	1.8	0.5160

[a,b] different letters indicate significant differences between lots.
[x,y] different letters indicate significant differences between treatments.
1: non-starter; 2: inoculated starter culture.
HPP-: non-treated; HPP+: pressurized.

Figure 1.

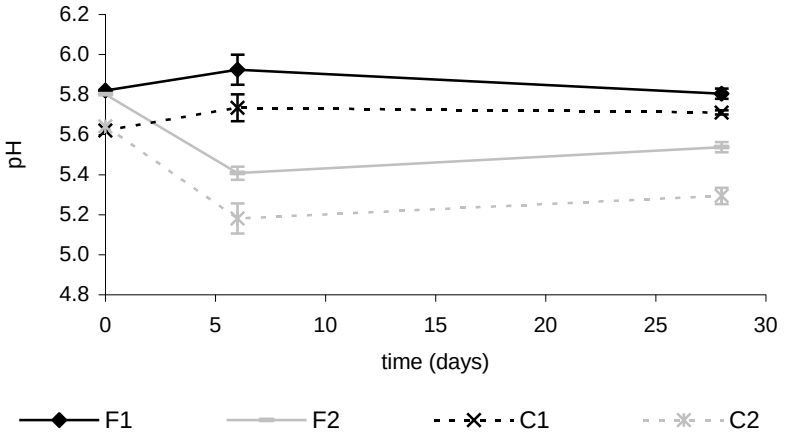
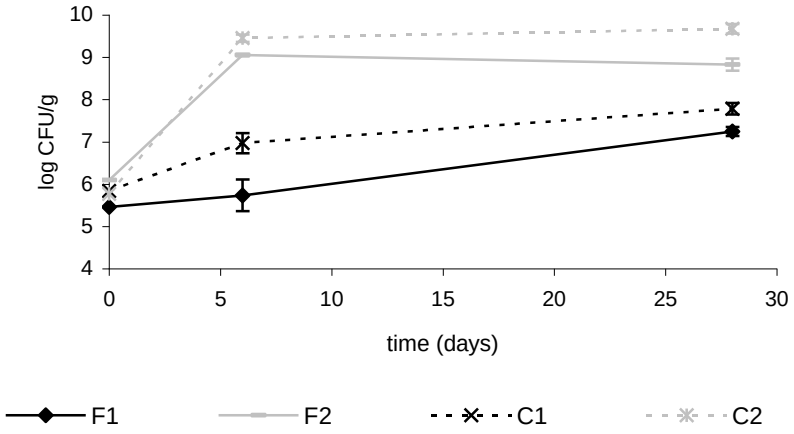
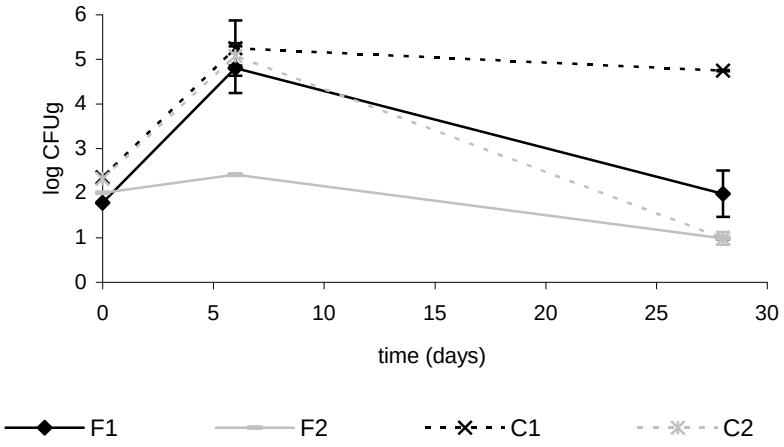


Figure 2.

a)



b)



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