Aminogenesis control in fermented sausages manufactured with pressurized meat batter and starter culture

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Abstract

The application of high hydrostatic pressure (200MPa) to meat batter just before sausage fermentation and the inoculation of starter culture were studied to improve the safety and quality of traditional Spanish fermented sausages (fuet and chorizo). Higher amounts of biogenic amines were formed in chorizo than in fuet. Without interfering with the ripening performance, hydrostatic pressure prevented enterobacteria growth but did not affect Gram-positive bacteria significantly. Subsequently, a strong inhibition of diamine (putrescine and cadaverine) accumulation was observed, but that of tyramine was not affected. The inoculated decarboxylase-negative strains, selected from indigenous bacteria of traditional sausages, were resistant to the HHP treatment, being able to lead the fermentation process, prevent enterococci development and significantly reduce enterobacteria counts. As a result, starter culture showed a protective effect against the accumulation of tyramine and both diamines, in sausages manufactured with either non-pressurized or pressurized meat batter.

Keywords: fermented sausages, high hydrostatic pressure, starter culture, biogenic amines, enterococci, enterobacteria

1. Introduction

Food quality and safety are of paramount importance to health and research organisations worldwide. The improvement of food products in relation to quality attributes arises from the requirement of good manufacturing practices and the need for minimising the risks, while ensuring the desired sensory traits of food products. Biogenic amines have been classically regarded as potentially hazardous microcomponents of food that may cause disorders to consumers, although the toxic doses and the mechanisms of such effects are not well established. Besides the toxicological implications, biogenic amines are of concern in relation to food hygiene (Mariné-Font, Vidal-Carou, Izquierdo-Pulido, Veciana-Nogués & Hernández-Jover, 1995). Biogenic amines accumulate in food as a consequence of bacterial amino acid-decarboxylase activity. Food produced through a fermentation process is described as particularly rich in biogenic amines. Indeed, the growth of a wide variety of bacteria potentially harbouring decarboxylase activity, the mild acidification and the proteolysis taking place during fermentation, are favourable conditions for biogenic amine accumulation. Fermenting microorganisms, mainly non-starter lactic acid bacteria, seem to play a significant role in the amine accumulation, especially tyramine. The contaminant microbial population (such as enterobacteria) also contributes largely to the occurrence of certain amines (such as diamines putrescine and cadaverine) being indicative of improper hygienic conditions. Therefore, the optimisation of hygienic conditions of both raw materials and processing is one of the key measures that enable the control of the aminogenesis during food processing and storage (Halász, Báráth, Simon-Sarkadi & Holzapfel, 1994; Bover-Cid & Holzapfel, 1999; Bover-Cid, Izquierdo-Pulido & Vidal-Carou, 2001).

The hygienic quality of raw materials may be improved by decreasing microbial loads through sterilization or pasteurisation, which is a common practice in the cheese making industry. However, in the case of fermented meat products, high temperatures cause detrimental changes in the raw materials, and thus, it is not possible to apply conventional heat treatments. Alternative non-thermal technologies show challenging possibilities in this connection. For instance, high hydrostatic pressure (HHP) is getting popularity especially in relation to the so called hurdle technology. Thanks to its advantages in comparison to thermal treatments to inactivate microorganisms with minimal sensory changes to the product, HHP has promising applications to satisfy consumer demand for high quality and safe meat products (Hugas, Garriga & Monfort, 2002). Some works have been published dealing with the effect of HHP on the stability of meat products and its biogenic amine content during storage (Ruíz-Capillas & Jiménez- Colmenero, 2004; Garriga, Marcos, Martín, Veciana-Nogués, Bover-Cid, Hugas & Aymerich, 2005). To the best of our knowledge, within the field of biogenic amines, the effect of HHP applied to raw materials has only been studied in milk used for cheese production as an alternative to pasteurization, with equivalent effects on aminogenesis (Novella-Rodríguez, Veciana-Nogues, Trujillo-Mesa & Vidal-Carou, (2002a). However, no research has been carried out in relation to fermented sausages.

Traditional Spanish low-acid ripened sausages are manufactured following traditional procedures, which are based on a spontaneous fermentation process at a relatively low temperature of approximately 10-15ºC. The ripening and drying process ensures low water activity values, but these slightly fermented products are characterised by a relatively high pH (over 5.3). Microflora contaminating raw materials (Gram-negative bacteria) may not be totally inhibited during the manufacture, compromising the safety and stability of the final product. The inoculation of

competitive and decarboxylase-negative starter culture has been shown to be a useful tool to inhibit spontaneous aminogenic microflora and thus considerably reduce aminogenesis (Bover-Cid, Hugas, Izquierdo-Pulido & Vidal-Carou, 2000a). However, the selection of appropriate strains is needed to keep the typical sensory characteristics of particular artisanal products (Di Maria, Basso, Santoro, Grazia & Coppola, 2002).

In this frame, the present work deals with the study of the potential application of mild HHP treatments on meat batter just before fermentation to improve the safety and quality of the final product. Moreover, decarboxylase-negative starter cultures, accurately selected from the indigenous microflora of traditional sausages showing optimal technological properties, were assessed in order to investigate their resistance to HHP and their ability to inhibit aminogenesis in two different types of traditional Spanish fermented sausages: fuet and chorizo.

2. Materials and methods

2.1. Sausage manufacture and sampling

The experiment was carried out with two types of traditional low-acid fermented sausages: Fuet and Chorizo. A total of 8 batches of fermented sausages were manufactured in parallel (following the experimental design of **Figure 1**) from the same lot of raw materials consisting of 50% of lean pork meat and 50% pork back fat. Meat raw materials were minced at -1ºC in a meat cutter (Tecmap, Barcelona, Spain), with an adjustable plate set at a hole diameter of 6 mm, and then mixed with other ingredients in a mixer machine (model 35P, Tecnotrip S.A., Terrassa, Spain). For fuet sausages the ingredients were 20 g/kg sodium chloride, 2.5 g/kg black pepper, 1.0 g/kg dextrose, 0.5 g/kg sodium ascorbate 0.1 g/kg potassium nitrate and 0.1 g/kg sodium nitrite.

Chorizo sausages contained 20 g/kg sodium chloride, 15 g/kg cayenne pepper, 15 g/kg paprika, 3.0 g/kg powdered garlic and 1.0 g/kg dextrose.

The mixture for each type of product was divided in two further parts. To one of them a mixture of bacteria consisting of two strains of *Lactobacillus sakei* (CTC6469 and CTC6626) and two strains of *Staphylococcus xylosus* (CTC6013 and CTC6169) was inoculated to achieve $4 \cdot 10^5$ CFU/g of sausage for each specie. These strains had previously been isolated from traditional low-acid fermented sausages. The other part was not inoculated in order to proceed with a spontaneous fermentation. Sausages were stuffed into collagen casings (Colex 32mm, Fibra S.A., Girona, Spain). For each type of product, either without or with starter culture, half of the stuffed sausages were submitted to a high hydrostatic pressure treatment of 200MPa for 10 min at 17ºC, using an industrial high hydrostatic pressurisation unit (Alstom, Nantes, France); whereas the other half were not pressurised. All sausages were hung in a climate chamber MLR.350 H (Sanyo Electric Co., Ora-Gun, Japan) at 12 ºC and with a relative humidity of >95% for 10 days and reduced to 80% till the end of the ripening process (21 days). Three sausages from each batch were sampled during the ripening process at selected times: just after stuffing (time 0) and after 1, 2 and 3 weeks.

2.2. Microbial analysis

After aseptically removing the casing, approximately 20 g of sausage were 10-fold diluted in buffered peptone water (AES Laboratories, Combourg, France) and homogenised in a Masticator (model 400, Cooke Laboratories, Alexandria, VA, USA) for 1 min. Serial of decimal dilutions were made and lactic acid bacteria (LAB) were enumerated by pour plating in Man, Rogosa and Sharpe (MRS) agar (Difco Laboratories, Detroit, Michigan, USA) at 30 °C for 72 h in anaerobiosis (Oxoid jars

with Anaero-Gen; Oxoid, Basingstoke, Hampshire, England), Gram-positive catalase potitive cocci (GCC+) by spread plating on mannitol salt agar (Difco Laboratories) at 30 °C for 48 h, enterococci by pour plating in kanamycin-esculin-azide agar (Oxoid LTD) at 37 °C for 24 h, *Enterobacteriaceae* by pour plating in violet red bile glucose agar (Merck, Darmstadt, Germany) with a double layer at 30 °C for 24 h.

The implantation of the inoculated strains and their dominance over the spontaneous flora were monitored by plasmid and RAPD profiling analysis as previously reported by Garriga et al. (2005).

2.3. Physico-chemical, nitrogenous fraction and biogenic amine analysis

Values of pH were determined using a Crison Basic 20 pH-meter by directly inserting an electrode into the sausage (model 52-32, Crison Instruments, S.A., Barcelona, Spain). Water activity was measured with the AquaLab® Series 3 Aw-meter (Decagon Devices Inc., Pullman, Washington, USA). Water content was measured gravimetrically, drying a sample aliquot to a constant weight at 102ºC (AOAC, 1995). To evaluate the proteolysis, total nitrogen was determined following the official Kjeldahl method in 2000 Kjeltec® equipment (Tecator, Foss España S.A., Barcelona, Spain). From a 0.6N perchloric extract of the sample without casing, the non-protein nitrogen was also determined by Kjeldahl and the free amino acid fraction (as alpha-amino nitrogen) by the Sorensen method through volumetric titration with 0.01N sodium hydroxide after reaction with formaldehyde (AOAC, 1995). The proteolysis index was calculated as the quotient between NPN and TN multiplied by 100 as described by Astiasarán, Villanueva and Bello (1990).

Biogenic amines (tyramine, histamine, putrescine, cadaverine, phenylethylamine, tryptamine, agmatine, spermidine and spermine) were extracted with

153 0.6N perchloric acid from spices (black pepper, cayenne pepper, paprika and powdered
154 garlic), raw meat batter and sausages without casings during ripening. Thereafter, they
155 were determined by ion-pair reverse-column high performance liquid chromatography
156 with post-column derivatization with *ortho*-phthalaldehyde according to the procedure
157 described by Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués and Vidal-Carou
158 (1996).

159 Due to the typical loss of water content during the manufacturing process, the
160 results of nitrogenous fractions and biogenic amine contents of samples, except for raw
161 materials, were referred to dry matter (dm).

162

163 *2.4. Statistical analysis*

164 Data was statistically treated using the SPSS 11.0 for Windows software (SPSS
165 Inc., Chicago, IL, USA) in order to determine the significance of the effect of starter
166 inoculation as well as the hydrostatic pressure treatment. A one-way analysis of the
167 variance (ANOVA) together with the post-hoc contrasts of Tuckey's HSD test was
168 applied to examine the differences among batches.

169

170 **3. Results and discussion**

171 *3.2. Microbial results*

172 Raw meat materials and spices used to manufacture the fuet and chorizo
173 sausages were examined for their microbiological quality. Bacterial counts
174 corresponding to meat batter (as the mixture of lean meat and back fat) were relatively
175 low, in log (CFU/g): 3.38 for LAB, 4.38 for GCC+, 2.68 log for enterococci and <2 for
176 enterobacteria, indicating a good hygienic quality of meat raw materials. Spices were

177 examined for the total mesophilic aerobic counts; high loads up to 7.4 log (CFU/g) were
178 found in black pepper and cayenne pepper, 6.2 log (CFU/g) in paprika and 4.6
179 log (CFU/g) in powdered garlic.

180 Changes in bacterial counts during sausage fermentation and ripening are shown
181 in **Table 1**. Due to starter inoculation, initial LAB and GCC+ counts were higher in
182 batches FS and CS than FnS and CnS. The implantation of the starter culture strains was
183 confirmed by plasmid and RAPD profile (data not shown). Maximum LAB counts were
184 reached after one and two weeks of ripening in starter (S) and spontaneously (nS)
185 fermented batches, respectively. GCC+ grew to a lesser extent than LAB, even in
186 batches where *Staphylococcus xylosus* strains had been inoculated as starters. After
187 ripening (21 days), irrespective of the type and starter inoculation, LAB counts were
188 over 8 logarithmic units, whereas GCC+ did not surpass 7.5 logs. Overall, the high
189 pressure processing of the sausages just after stuffing did not influence the initial LAB
190 and GCC+ counts significantly or their progression during the manufacture in any of the
191 products without or with starter culture inoculation.

192 The behaviour of enterococci during fermentation and ripening was similar in
193 fuet and chorizo batches. Thus, spontaneously fermented products showed increasing
194 loads of enterococci during the first week and then remained around 10^4 CFU/g. No
195 effect by the HHP processing was observed. By contrast, the starter inoculation
196 prevented enterococci development significantly remaining around 10^2 CFU/g
197 throughout the ripening.

198 More important and significant differences in relation to the occurrence of
199 enterobacteria were found among all the batches. On the one hand, non-pressurised
200 spontaneously fermented sausages showed a notable increase of enterobacteria loads
201 during the first week of fermentation, in fuet (FnS-nP) being slightly lower than in

chorizo (CnS-nP). Thereafter, a decrease was observed to $<10^2$ CFU/g in fuet and to $5 \cdot 10^3$ CFU/g in chorizo at the end of the ripening. HHP treatment inhibited enterobacteria growth in fuet (FnS-P), in which they were below the detection limit throughout the ripening, and significantly reduced its development in chorizo (CnS-P). Nevertheless, starter cultures were much more effective in inhibiting enterobacteria growth, since they quickly decreased not only in fuet (FS-nP and FS-P) but also in chorizo (CS-nP and CS-P) sausages.

3.2. Physico-chemical and proteolysis related parameters

Figure 2 shows the pH and Aw values of sausages during the production process. Initial pH values were within the normal range (Ordóñez, Hierro, Bruna, & Hoz, 1999). Spontaneously fermented batches showed a weak acidification without statistically significant effect due to HHP application. Chorizo sausages showed slightly lower pH values than fuet, which could be related to an extra amount of fermentable carbohydrates coming from paprika added to chorizo (Lois, Gutiérrez, Zumalacárregui & López, 1987). The inoculation of the starter resulted in a much stronger acidification during the first week of production, again to lower pH values in chorizo than in fuet. Then, a pH increase of 0.35 – 0.42 units occurred and as a consequence final pH values were not significantly different from the corresponding spontaneously fermented batches. The HHP treatment did not seem to affect the fermentative activity of either the spontaneous microflora or the starter culture, and the course of acidification was the same between non-pressurised and pressurised sausages.

Values of Aw decreased gradually during the first two weeks and more intensively during the last week of the ripening, reaching final values lower than 0.85 in

all products. No significant differences were found between products (fuet and chorizo), by the inoculation of starters or by HHP treatment. The same can be said regarding water content decrease (from 62.5% to 36.7% on average). Since all samples were hung in the same climatic chamber under the same environmental conditions, it can be concluded that the drying process was not affected by the different formulation (type of product), the inoculation of starter cultures or the high pressure processing.

The evolution of the proteolysis related parameters was affected by the type of product, the inoculation of starter culture as well as the application of HHP, but in a different manner depending on the parameter (**Table 2**). The PI, as the percentage of NPN among total nitrogen, did not increase significantly during manufacture, and the starter culture had little influence. By contrast, HHP treatment resulted in higher values of PI, which was especially evident for chorizo sausages. However, differences in the overall PI among the four batches of each product were not statistically significant according to the *post-hoc* contrasts of the ANOVA test (HSD of Tuckey). Concerning the content free amino acids, values of AAN increased gradually throughout the ripening, to a higher rate in chorizo sausages in comparison with fuet. Although batches with starter cultures tended to show higher AAN values than those spontaneously fermented, differences were never statistically significant. Neither did the HHP seem to exert any effect on AAN release. The proteolysis occurring during meat fermentation is a rather complicated phenomenon involving several types of endogenous and microbial enzymes (Ordóñez, Hierro, Bruna & Hoz, 1999). The respective roles have been a source of controversy, but numerous studies over the last decade have concluded that muscle proteinases (particularly cathepsin D) are activated by the drop of pH and seem primarily responsible for proteolysis during the early fermentation, while bacterial enzymes are more important during the latter stages of ripening (Molly, Demeyer,

252 Johansson, Raemaekers, Ghistelinck & Geenen, 1997; Hughes, Kerry, Arendt,
253 Kenneally, McSweeney & O'Neill, 2002). It has been reported that high pressure up to
254 400 MPa may induce proteolysis due to lysosomal membrane breakdown with the
255 consequent release of proteases into the cytosol and, in turn, the activation of some
256 cathepsins (Homma, Ikeuchi, & Suzuki, 1994; Jung, Lamballerie-Anton, Taylor &
257 Ghoul, 2000). In the present study, although the PI values show a tendency to be higher
258 in pressurized batches when compared to non-treated ones, nothing can be stated about
259 the effect of HHP (200 MPa) on the proteolytic changes during the ripening of fuet and
260 chorizo.

261 The high pressure processing of the meat batter did not significantly affect the
262 ripening performance, since no significant differences were observed in the pH, Aw and
263 proteolysis. Moreover, the colour of the sausages was not visually affected by the
264 pressure treatment applied.

265

266 *3.3. Aminogenesis*

267 Contents of biogenic amines of raw materials are shown in **Table 3**. In meat
268 batter, the only amines present in significant amounts were the physiological
269 polyamines spermidine and spermine, which conforms with high hygienic quality of
270 meat used for sausage elaboration, as do the microbial counts. Other biogenic amines
271 were found in the spices. In the particular case of powdered garlic, added in chorizo
272 manufacture, considerable levels of tyramine and lower levels of phenylethylamine
273 were detected. However, the final quantitative contribution of these spices to the total
274 biogenic amine pool in the stuffed sausage was insignificant (always below 0.05 mg/kg
275 to the final mixture), since they are incorporated in low concentrations from 2.5 g/kg to
276 15 g/kg. On the other hand, the aerobic counts in spices ranged from 4.6 to

7.4 log (CFU/g), and thus their contribution to total bacterial load of the meat batter might be calculated to range from 2 to 5 log (CFU/g) depending on the spices. The occurrence of biogenic amines (especially aromatic amines and cadaverine) in spices may be indicative of contamination with amino acid-decarboxylase positive microorganisms. In this sense, spices might have been vehicles of potentially aminogenic microorganisms to meat batter or eventually amino acid-decarboxylase enzymes, which might have contributed to biogenic amine accumulation during the subsequent fermentation and ripening process.

During sausage manufacture, contents of physiological polyamines did not show significant changes ($p>0.05$). No influence of starter inoculation or HHP processing was observed in either type of product, fuet or chorizo (**Figure 3**). These data are in agreement with the hypothesis that spermidine and spermine in meat products are of endogenous origin, not being formed by microbial activity.

By contrast, the main biogenic amines associated with bacterial activity in fermented meat products (tyramine, putrescine and cadaverine) were influenced by all three variables studied (product type, starter culture and HHP treatment), in a different manner depending on the amine (**Figure 4**). Batches without starter culture and no HHP treatment (that is, FnS-nP and CnS-nP) can be considered as "control", from which the influence of formulation on the quantitative and qualitative aspects of aminogenesis can be determined. Biogenic amine accumulation was much lower in fuet that in chorizo sausages. Tyramine was the only biogenic amine detected in fuet, whereas cadaverine was the major amine in chorizo sausages, which is usually associated with lysine-decarboxylase activity of undesirable gram-negative bacteria (Bover-Cid & Holzapfel, 1999; Bover-Cid, Miguélez-Arrizado, Latorre-Moratalla & Vidal-Carou, 2005). Tyramine was the second amine, followed by putrescine. No other biogenic amine

(histamine, phenylethylamine or tryptamine) was detected in any sample. Several explanations can be made to account for the differences in the biogenic amine accumulation between products. For the one hand, the spices added in chorizo (mainly garlic, but also cayenne pepper and paprika) may have been a vehicle of aminogenic contaminant bacteria or decarboxylases enzymes. Another difference between products was the amount of curing agents, since fuet contained up to twice the initial nitrate and nitrite content in comparison to chorizo, in which 0.05 g/kg nitrate and 0.04 g/kg nitrite were incorporated as constituents of cayenne pepper and paprika (Garriga et al., 2005). Under these concentrations bacteria are less inhibited in chorizo than in fuet. Moreover, chorizo reached lower pH values and higher free amino acid contents during fermentation. Both factors are known to favour biogenic amine production by microorganisms, since bacterial decarboxylase enzymes are induced by the presence of precursor amino acids at mild acid pH (Bover-Cid & Holzapfel, 1999). Nevertheless, the extremely low levels of biogenic amines in fuet sausages were surprising in comparison to the variable but higher levels (140 mg/kg on average with a relative standard deviation of 73%) usually reported for similar products (Miguélez-Arrizado, Bover-Cid, Latorre-Moratalla & Vidal-Carou, 2005). In a previous work (Bover-Cid et al., 2005) the aminogenesis in spontaneously fermented fuet was much more important, even when the hygienic quality of raw materials was optimal in both cases. In this cited work, the temperature of fermentation was considerably higher (17 ºC) than in the present study (12 ºC), and this may suggest that, besides the hygiene of raw materials and formulation, temperature might be a technologically important parameter to control the aminogenic activity of spontaneous fermenting microorganisms.

Low contents of biogenic amines were also observed in the other three batches of fuet manufactured, which only allows to corroborate that starter cultures kept unable

to produce biogenic amines under *in situ* sausage fermentation environment, and HHP treatment did not have any influence. Therefore, the protective effect of HPP processing and indigenous starter culture will be discussed based on the results obtained for chorizo sausages. In spontaneously fermented sausages, the application of HPP (batch CnS-P) resulted in a strong inhibition of diamine accumulation, the levels of putrescine and cadaverine being up to 88 % and 98 % lower than the non-pressurised batch (CnS-nP). By contrast, tyramine production was almost equal in both batches. It seems that high pressure (at 200MPa) has a hygenising effect reducing the lysine- and ornithine-decarboxylase activity of contaminant bacteria in agreement with the also reduced counts of enterobacteria; whereas tyrosine-decarboxylase positive microorganisms, such as enterococci, are not sensitive to the applied pressure.

Little is known about the effect of HPP treatment of raw materials on the aminogenesis occurring during food fermentation. Some reports have been published dealing with cheese making. Milk pressurization at 500 MPa for 15 min at 20 ºC was equivalent to heat pasteurization (72 ºC for 15 seg), without differences on biogenic amine accumulation (Novella-Rodríguez et al., 2002a). The application of 400 MPa for 5 min to dried curds after salting in brine, in order to accelerate cheese ripening, had no significant effect on aminogenesis in comparison to untreated samples. However, milder and longer high-pressure treatment (50 MPa for 72 h) yielded almost 3-fold higher tyramine contents (Novella-Rodríguez, Veciana-Nogués, Saldo & Vidal-Carou, 2002b).

The inoculation of strains previously selected among indigenous sausage microflora as starter culture was the most protecting measure to avoid biogenic amine accumulation during chorizo manufacture. Indeed, starters were not only able to reduce up to 93 % putrescine and up to 99 % cadaverine accumulation, but also about 76 % of tyramine. Starter cultures are not always reported as being able to reduce or inhibit the

352 accumulation of all biogenic amines, which have been attributed to their low
353 competitiveness or difficulty to adapt to meat fermentation (Bover-Cid et al., 2000a). If
354 starters are accurately selected among decarboxylase-negative strains isolated from
355 fermented sausages, the probability of success is much higher. As reported in a previous
356 work (Bover-Cid, Izquierdo-Pulido & Vidal-Carou, 2000b), mixed starter cultures of *L.*
357 *sakei* (strain CTC494) with *S. xylosus* (strain CTC3037 or CTC3050) were effective in
358 reducing 90% of the overall aminogenesis in fuet. The results obtained proved the
359 suitability of the selected indigenous starters for both types of fermented product (fuet
360 and also chorizo).
361

362 **4. Conclusion**

363 The high pressure treatment (200MPa for 10 min at 17 ºC) applied to meat batter
364 showed a strong inhibitory effect on diamine formation, but hardly any influence on
365 tyramine accumulation. Moreover, high hydrostatic pressure processing before sausage
366 fermentation did not reduce the capability of the inoculated lactobacilli and
367 staphylococci strains to lead the fermentation, which wielded a strong protective effect
368 against tyramine and diamine producing microflora. The pressurization of meat batter
369 did not interfere with the ripening performance, since no significant differences were
370 observed in pH, Aw, proteolysis and in the colour of the sausages. Therefore, it seems
371 challenging and interesting to proceed with further research dealing with such non-
372 thermal technology to improve the hygienic status of raw material.
373

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456

Figure captions

Figure 1. Experimental design for the study of aminogenesis in Fuet and Chorizo sausages manufactured through spontaneously and starter mediated fermentation, without and with high hydrostatic pressure treatment.

Figure 2. Changes in pH (top) and water activity (bottom) during the manufacture of Fuet (F, left column) and Chorizo (C, right column) through spontaneously (nS) and starter (S) mediated fermentation, without (nP) and with (P) high hydrostatic pressure treatment.

Figure 3. Polyamine contents in Fuet and Chorizo sausages manufactured through spontaneously and starter mediated fermentation, without and with high hydrostatic pressure treatment.

Figure 4. Changes in tyramine, putrescine and cadaverine contents during the manufacture of Fuet (F, left column) and Chorizo (C, right column) through spontaneously (nS) and starter (S) mediated fermentation, without (nP) and with (P) high hydrostatic pressure treatment.

Table 1. Microbial counts[a], log (CFU/g), during the manufacture of Fuet and Chorizo through spontaneously and starter mediated fermentation, without and with high hydrostatic pressure treatment.

	Fuet (F)				Chorizo (C)			
	Control - spontaneous fermentation (nS)		Starter (S)		Control - spontaneous fermentation (nS)		Starter (S)	
	Not pressurized	Pressurized (P)	Not pressurized	Pressurized (P)	Not pressurized	Pressurized (P)	Not pressurized	Pressurized (P)
Day	**FnS-nP**	**FnS-P**	**FS-nP**	**FS-P**	**CnS-nP**	**CnS-P**	**CS-nP**	**CS-P**
LAB								
0	3.47 *(0.13)*	3.47 *(0.13)*	5.70 *(0.13)*	5.70 *(0.13)*	3.65 *(0.41)*	3.65 *(0.41)*	5.65 *(0.14)*	5.65 *1.38*
7	8.29 *(0.03)*	8.27 *(0.02)*	9.39 *(0.45)*	8.96 *(0.04)*	8.26 *(0.07)*	8.41 *(0.03)*	9.54 *(0.05)*	8.98 *1.45*
13	8.81 *(0.09)*	8.80 *(0.04)*	9.14 *(0.14)*	9.16 *(0.07)*	9.03 *(0.16)*	9.12 *(0.10)*	9.59 *(0.05)*	9.74 *(0.03*
21	8.18 *(0.23)*	8.74 *(0.15)*	8.78 *(0.15)*	8.70 *(0.07)*	8.66 *(0.11)*	8.95 *(0.14)*	9.31 *(0.11)*	9.51 *(0.13*
GC+								
0	4.04 *(0.64)*	4.09 *(0.64)*	5.55 *(0.10)*	5.60 *(0.10)*	3.16 *(0.28)*	3.68 *(0.28)*	6.58 *(0.08)*	5.73 *1.16*
7	5.49 *(0.29)*	5.49 *(0.64)*	6.05 *(0.31)*	5.53 *(0.15)*	5.79 *(0.55)*	6.75 *(0.22)*	7.04 *(0.26)*	6.51 *(0.33*
13	6.21 *(0.07)*	6.99 *(0.42)*	7.30 *(0.46)*	6.84 *(0.79)*	6.99 *(0.13)*	7.37 *(0.24)*	7.32 *(0.37)*	6.44 *(0.70*
21	6.15 *(0.26)*	7.41 *(0.81)*	7.40 *(0.29)*	6.86 *(0.19)*	6.68 *(0.06)*	7.42 *(0.14)*	7.13 *(0.47)*	6.67 *(0.39*
Enterococci								
0	2.64 *(0.06)*	2.66 *(0.06)*	2.48 *(0.13)*	2.29 *(0.13)*	2.66 *(0.47)*	4.62 *(0.47)*	2.90 *(0.39)*	2.59 *(0.26*
7	4.62 *(0.24)*	4.75 *(0.16)*	2.06 *(0.21)*	2.20 *(0.17)*	4.73 *(0.21)*	4.57 *(0.30)*	2.37 *(0.17)*	2.33 *(0.43*
13	4.78 *(0.76)*	4.26 *(0.20)*	2.13 *(0.35)*	2.28 *(0.20)*	4.62 *(0.49)*	4.63 *(0.31)*	2.22 *(0.17)*	2.16 *(0.28*
21	3.79 *(0.28)*	4.32 *(0.17)*	2.14 *(0.08)*	1.88 *(0.25)*	4.19 *(0.41)*	4.79 *(0.18)*	2.50 *(0.05)*	2.33 *(0.05*
Enterobacteria								
0	<2	<2	<2	<2	<2	<2	<2	<2
7	5.60 *(1.10)*	<2	<2	<2	6.62 *(0.44)*	3.60 *(0.59)*	<2	<2
13	4.51 *(1.24)*	<2	<2	<2	5.67 *(1.10)*	3.63 *(0.95)*	<2	<2
21	<2	<2	<2	<2	3.52 *(0.66)*	2.48 *(1.36)*	<2	<2

[a]Data is expressed as the mean and in italics the standard deviation of the three replicates.

Table 2. Results[a] on proteolytic related parameters (alpha-amino nitrogen, NAA; non-protein nitrogen, NPN; proteolysis index, IP) during the manufacture of Fuet and Chorizo through spontaneously and starter mediated fermentation, without and with high hydrostatic pressure treatment.

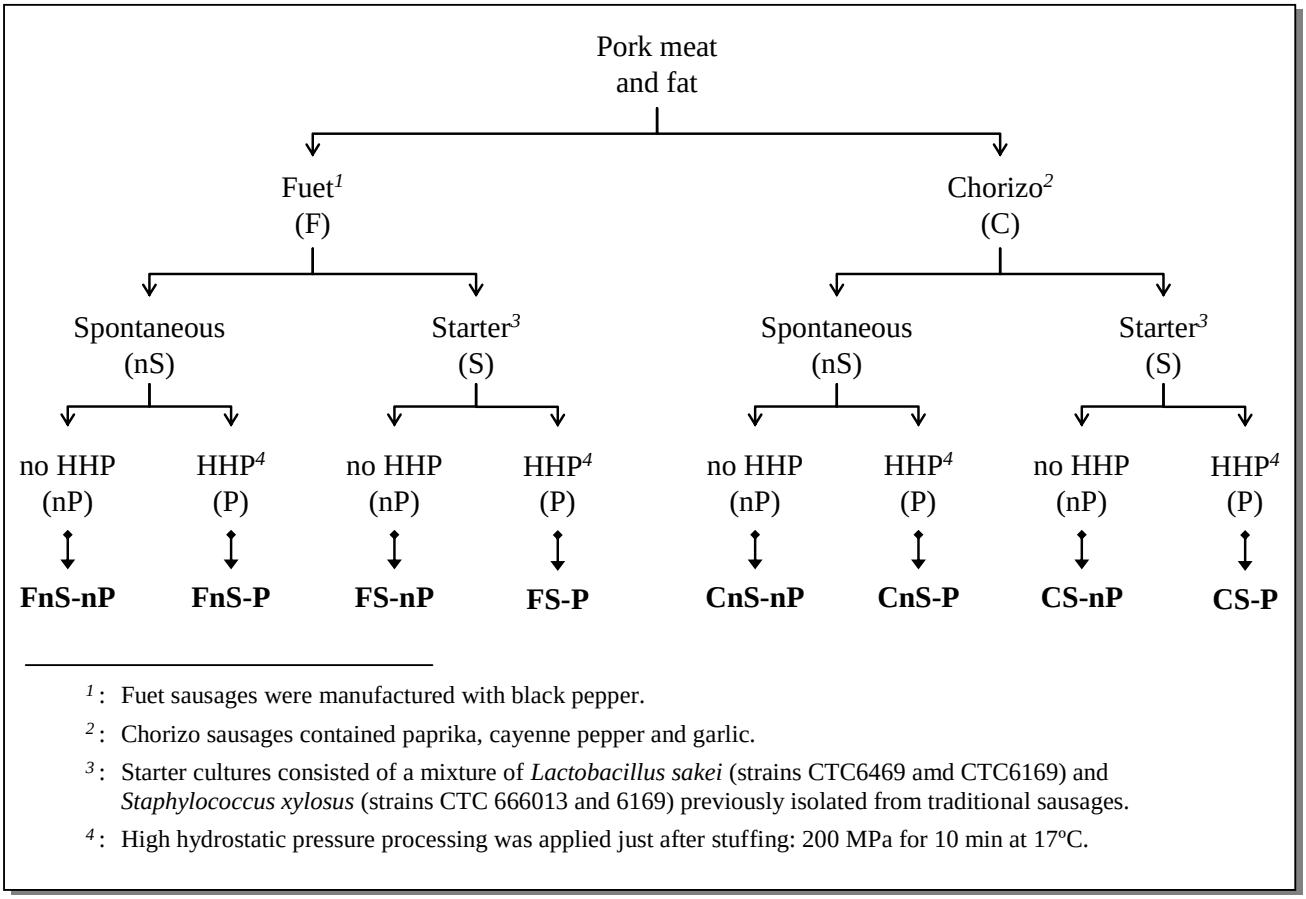
	Fuet (F)				Chorizo (C)			
	Spontaneous fermentation (nS)		Starter (S)		Spontaneous fermentation (nS)		Starter (S)	
	Not pressurized (nP)	Pressurized (P)	Not pressurized (nP)	Pressurized (P)	Not pressurized (nP)	Pressurized (P)	Not pressurized (nP)	Pressurized (P)
Day	**FnS-nP**	**FnS-P**	**FS-nP**	**FS-P**	**CnS-nP**	**CnS-P**	**CS-nP**	**CS-P**
AAN (mg/g dw)								
0	1.10 *(0.11)*	1.41 *(0.10)*	1.23 *(0.05)*	1.41 *(0.12)*	1.28 *(0.17)*	1.58 *(0.28)*	1.37 *(0.09)*	1.66 *(0.19)*
7	1.74 *(0.27)*	1.60 *(0.10)*	2.56 *(0.69)*	2.02 *(0.20)*	2.06 *(0.06)*	2.19 *(0.22)*	2.40 *(0.07)*	2.46 *(0.14)*
13	2.99 *(0.62)*	1.64 *(0.04)*	2.32 *(0.19)*	2.41 *(0.20)*	2.64 *(0.06)*	2.64 *(0.03)*	3.11 *(0.18)*	2.90 *(0.11)*
21	1.97 *(0.26)*	2.08 *(0.10)*	1.92 *(0.15)*	2.51 *(0.02)*	3.12 *(0.32)*	3.07 *(0.18)*	2.83 *(0.09)*	3.42 *(0.18)*
NPN (mg/g dw)								
0	1.20 *(0.75)*	1.83 *(0.45)*	1.44 *(0.28)*	2.74 *(0.88)*	3.23 *(0.50)*	4.01 *(2.26)*	4.06 *(0.58)*	4.60 *(1.08)*
7	1.14 *(0.68)*	1.60 *(0.10)*	3.64 *(2.94)*	2.28 *(1.21)*	7.10 *(3.48)*	5.66 *(0.27)*	6.05 *(1.15)*	4.22 *(1.31)*
13	0.86 *(0.56)*	2.25 *(0.21)*	1.61 *(1.01)*	2.70 *(0.58)*	2.92 *(1.01)*	3.73 *(0.41)*	0.72 *(0.21)*	2.91 *(0.63)*
21	2.17 *(1.18)*	3.55 *(0.68)*	1.86 *(0.47)*	3.76 *(0.54)*	4.07 *(1.30)*	4.56 *(1.98)*	3.73 *(1.64)*	5.28 *(0.06)*
PI (%)								
0	1.54 *(0.14)*	2.54 *(0.60)*	1.48 *(0.09)*	3.31 *(1.12)*	1.84 *(0.16)*	5.68 *(2.91)*	2.14 *(0.11)*	7.16 *(1.43)*
7	2.36 *(0.37)*	2.12 *(0.05)*	3.16 *(0.85)*	2.86 *(1.58)*	2.77 *(0.02)*	7.66 *(0.43)*	3.41 *(0.28)*	5.73 *(1.57)*
13	4.06 *(0.69)*	2.99 *(0.22)*	2.88 *(0.28)*	3.27 *(0.67)*	3.31 *(0.01)*	4.77 *(0.53)*	4.23 *(0.14)*	4.06 *(0.96)*
21	2.62 *(0.13)*	4.45 *(0.75)*	2.37 *(0.10)*	5.07 *(1.11)*	3.65 *(0.31)*	5.72 *(2.21)*	3.76 *(0.07)*	6.96 *(0.34)*

[a]Data is expressed as the mean and in italics the standard deviation of the three replicates.

Table 3. Mean (*standard deviation*) values of biogenic amine contents (mg/kg fresh matter) of spices and raw meat batter used for sausage manufacturing.

	Black pepper	**Paprika**	**Cayenne pepper**	**Powdered garlic**	**Meat batter**
Tyramine	3.69 (*0.12*)	3.60 (*0.38*)	0.48 (*0.01*)	15.76 (*0.12*)	<0.3
Phenylethylamine	nd	nd	nd	2.03 (*0.18*)	nd
Putrescine	nd	5.40 (*0.18*)	2.92 (*0.28*)	10.23 (*0.27*)	nd
Cadaverine	2.50 (*0.09*)	3.34 (*0.12*)	<0.3	2.31 (*0.08*)	nd
Agmatine	nd	4.48 (*0.25*)	7.79 (*0.44*)	3.10 (*0.09*)	nd
Spermidine	0.76 (*0.15*)	6.34 (*0.30*)	4.00 (*0.82*)	32.72 (*0.02*)	2.82 (*0.38*)
Spermine	5.04 (*0.66*)	8.70 (0.38)	4.13 (*1.05*)	20.49 (*0.13*)	24.26 (*3.64*)

Figure 1.



[1]: Fuet sausages were manufactured with black pepper.

[2]: Chorizo sausages contained paprika, cayenne pepper and garlic.

[3]: Starter cultures consisted of a mixture of *Lactobacillus sakei* (strains CTC6469 amd CTC6169) and *Staphylococcus xylosus* (strains CTC 666013 and 6169) previously isolated from traditional sausages.

[4]: High hydrostatic pressure processing was applied just after stuffing: 200 MPa for 10 min at 17ºC.

Figure 2.

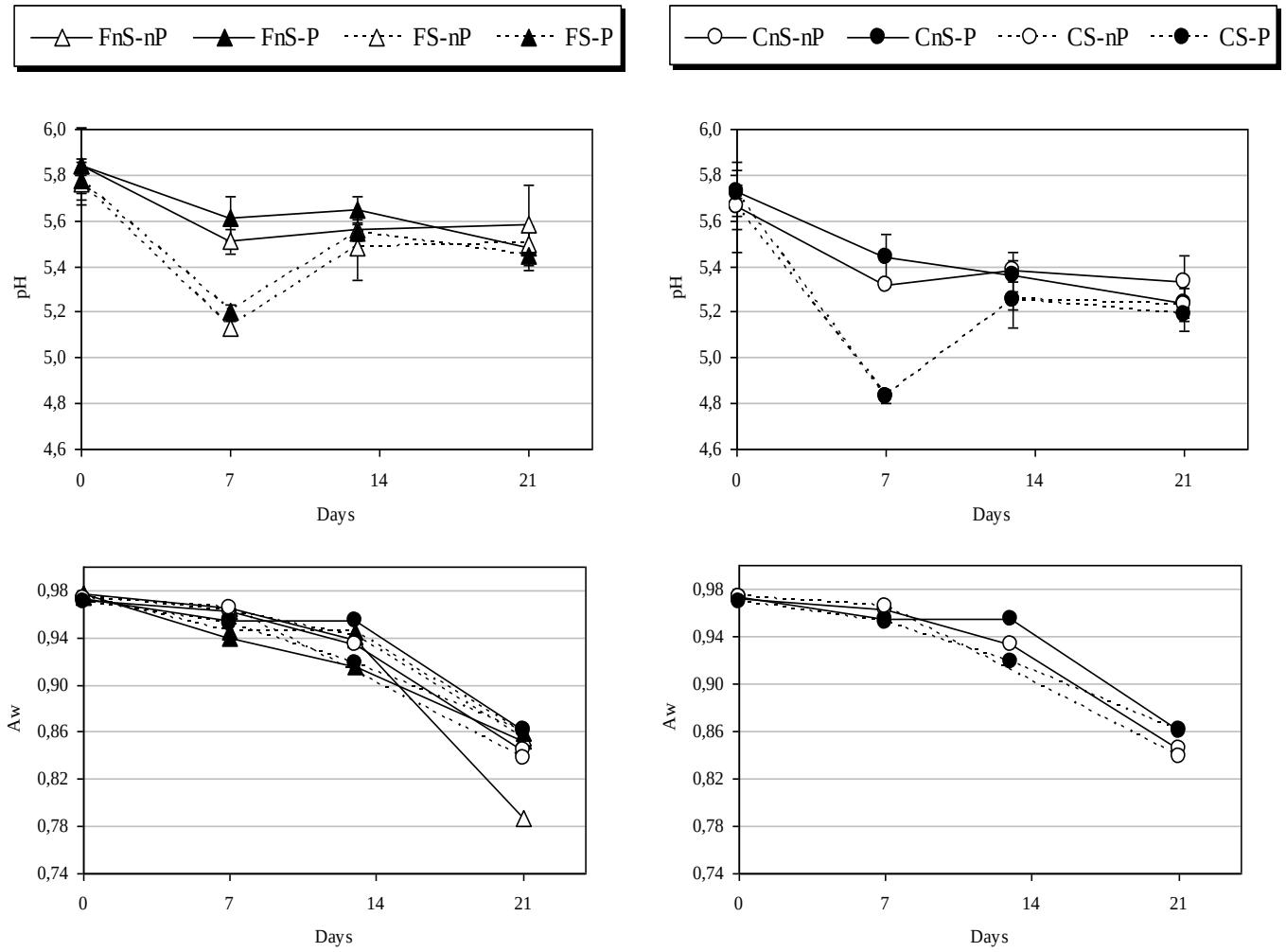

FnS-nP
FnS-P
FS-nP
FS-P
CnS-nP
CnS-P
CS-nP
CS-P
pH
Aw
Days
6,0
5,8
5,6
5,4
5,2
5,0
4,8
4,6
0,98
0,94
0,90
0,86
0,82
0,78
0,74
0
7
14
21

Figure 3.

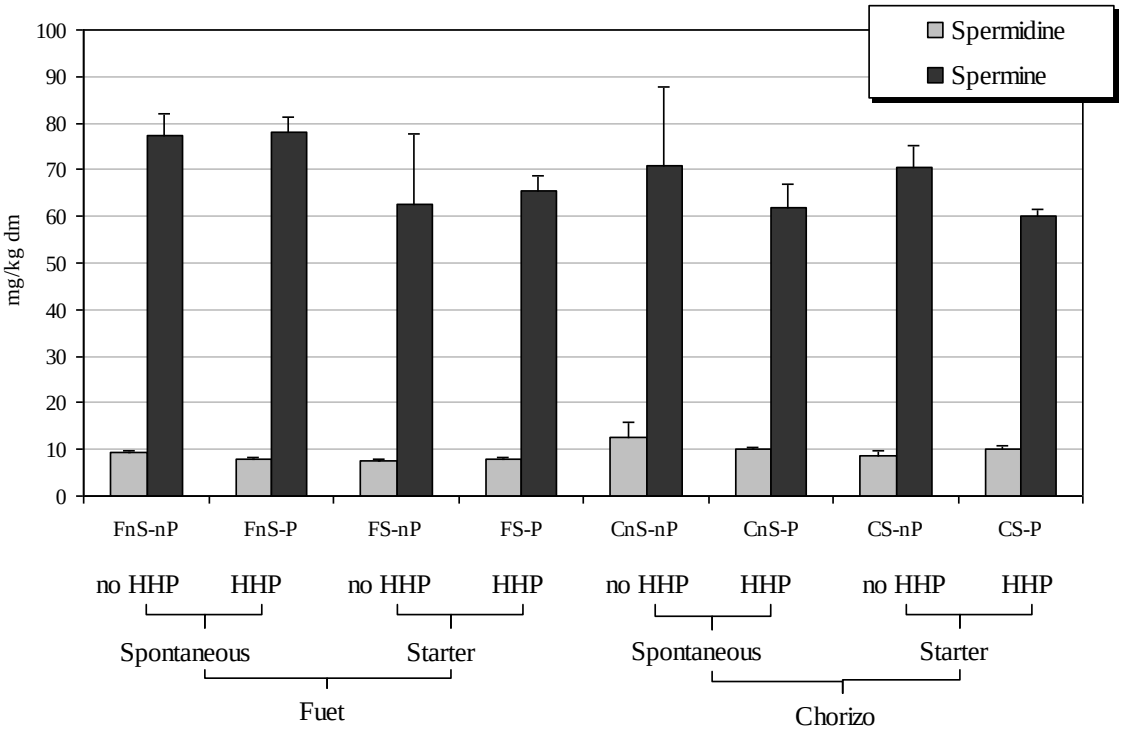
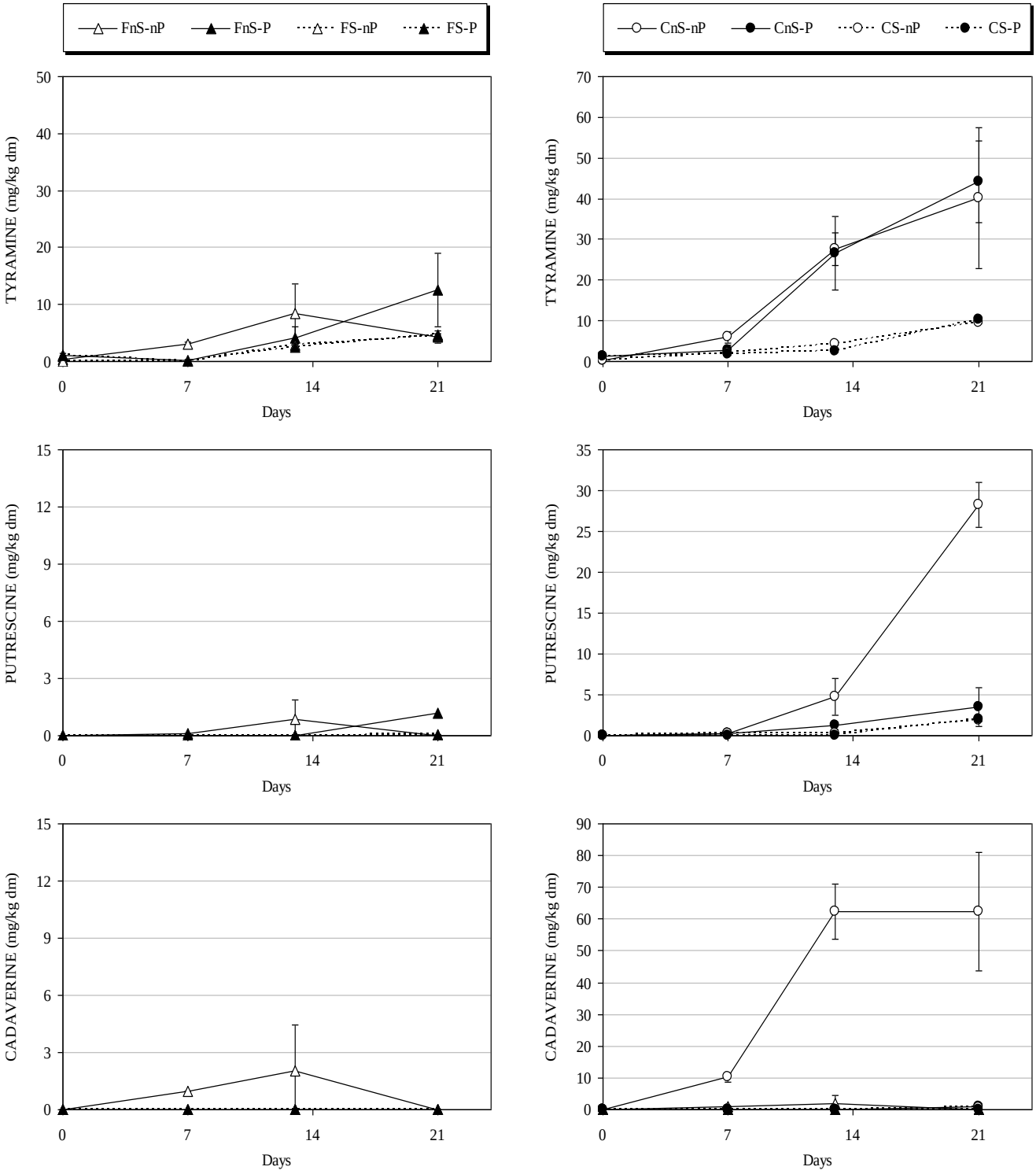

Spermidine
Spermine
mg/kg dm
100
90
80
70
60
50
40
30
20
10
0
FnS-nP
FnS-P
FS-nP
FS-P
CnS-nP
CnS-P
CS-nP
CS-P
no HHP
HHP
no HHP
HHP
no HHP
HHP
no HHP
HHP
Spontaneous
Starter
Spontaneous
Starter
Fuet
Chorizo

Figure 4.


FnS-nP
FnS-P
FS-nP
FS-P
CnS-nP
CnS-P
CS-nP
CS-P
TYRAMINE (mg/kg dm)
PUTRESCINE (mg/kg dm)
CADAVERINE (mg/kg dm)
Days
0
7
14
21