Effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *Debaryomyces hansenii yeast*

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Abstract

The inoculation of a *Debaryomyces hansenii* strain in dry fermented sausages with reduced fat and salt contents was evaluated in terms of chemical, microbial and consumer acceptability. The implantation of the inoculated yeast strain was confirmed by RAPDs of M13 minisatellite. A reduction of 17-20 % salt and 10-16 % fat content was achieved. These reductions affected the sausage quality by producing an increase in a_w, hardness and chewiness values and a decrease of staphylococci growth. However, *D. hansenii* inoculation compensated these changes although it was not able to modify neither the hardness of reduced fat batches nor the staphylococci growth decrease. In terms of sensory acceptability, different preferences patterns of consumers were found. Yeast inoculation improved the aroma and taste quality when fat or salt reductions were carried out in dry fermented sausages.

Keywords: fat, salt, sensory, *Debaryomyces hansenii*, dry fermented sausage, yeast.

1. Introduction

The intake of meat products has grown around 10% in industrialized countries in spite of their fat and salt content (WHO/FAO, 2003). However, during the last years a demand for low fat and salt food has grown (Ruusunen & Puolanne, 2005). Therefore, meat products such as dry fermented sausages are being reformulated to adjust their fat and salt content (Aaslying, Vestergaard & Koch, 2014; Beriain, Gómez, Petri, Insausti & Sarriés, 2011). However, these ingredients cannot be reduced without affecting organoleptic and technologic characteristics. On the one hand, fat contributes to nutritional (source of essential fatty acids, liposoluble vitamins and energy), organoleptic (flavour, texture, mouthfeel) and technological properties (release of moisture) (Olivares, Navarro, Salvador & Flores, 2010). On the other hand, salt is also involved in organoleptic (flavour, texture) and technologies properties (myofibrillar protein solubilization, a_w decrease) (Corral, Salvador & Flores, 2013).

Different strategies have been studied to reduce fat and salt content in dry fermented sausages, since preservation of product acceptability is a decisive criterion when developing this kind of products (Wirth, 1988). Fat content has been replaced by soy oil (Muguerza, Ansorena & Astiasarán, 2003), olive oil (Bloukas, Paneras & Fournitzis, 1997), konjac gel (Ruíz-Capillas, Triki, Herrero, Rodriguez-Salas & Jiménez-Colmenero, 2012), inulin (Mendoza, García, Casas & Selgas, 2001), and fibres (Salazar, García, & Selgas, 2009). However, few studies have dealt with fat reduction without replacers and its effect on sensory characteristics. Generally, fat reduction (10 %) affected the external appearance and flavour intensity of fermented sausages (Liaros, Katsanidis & Bloukas, 2009) while higher reduction percentages (20 %) produced a suitable acceptability (Papadima & Bloukas, 1999 and Olivares et al. 2010); in spite of a lowest taste, aroma and hardness (Olivares et al., 2010 and 2011) and appearance (Papadima & Bloukas, 1999) perceived by consumers.

Regarding salt reduction, different salts have been used as NaCl substitute (Corral et al., 2013; Gimeno, Astiasarán & Bello, 1999) although they did not achieve

57 sensory acceptable products (Gimeno et al., 1999). Major salt reductions (40-50%) had
58 a negative effect on many sensory characteristics such as hardness, bitterness, aroma
59 and taste acceptability (Gelabert, Gou, Guerrero & Arnau, 2003; Campagnol, dos
60 Santos Wagner, Terra & Pollonio, 2011). With small salt reductions (16 %), the best
61 results were found when KCl alone was used even though the aroma acceptability was
62 still affected (Corral et al., 2013).

63 The effect of fat and salt reductions together in dry fermented sausages has
64 been scarcely studied. A strategy of combining a reduction in the salt content and a
65 simultaneous modification in the lipid fraction using olive or linseed oils resulted in
66 products with lowest sodium content, highest calcium content and a significant supply
67 of omega-3 fatty acids (García-Iñiguez de Ciriano, Berasategi, Navarro-Blasco,
68 Astiasarán & Ansorena, 2013) and highest MUFA content (Beriain et al., 2011)
69 producing an improved nutritional sausage profile. Both studies indicated a sensory
70 acceptable characteristic of the new formulations although differences in texture and
71 taste were reported by the trained panel.

72 The use of starter yeasts can be an alternative to improve the sensory
73 characteristics of the dry fermented sausages. *Debaryomyces hansenii* is the
74 predominant yeast which proliferates in dry fermented sausage environment (Cocolin,
75 Urso, Rantsiou, Cantoni & Comi, 2006). The growth of *D.hansenii* in the sausage
76 surface can control water release in low fat sausages thus improving sausage aroma
77 lost by salt reduction as reported by Campagnol et al. (2011) who tried to improve the
78 sensory characteristic by the addition of a yeast extract (*Saccharomyces cerevisiae*).
79 The mechanisms behind aroma loss due to salt reduction are probably due to the
80 salting out effect that salt produces on volatile compounds (Desmond, 2006) in addition
81 to the effect of salt on the biochemical reactions involved in aroma generation.

82 Nevertheless, important differences have been observed when different yeasts
83 strains are inoculated in fermented sausages (Olesen, & Stahnke, 2000; Andrade,
84 Córdoba, Sánchez, Casado & Rodríguez, 2009). Recently, Cano-García, Flores &

Belloch (2013) isolated *D. hansenii* strains from traditional fermented sausages and reported their aroma potential using a meat model system (Cano-García, Rivera-Jiménez, Belloch & Flores, 2014). Therefore, the aim of this work was to elucidate the effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with *Debaryomyces hansenii*, since this yeast could offset the quality defects produced by fat and/or salt reductions.

2. Materials and methods

2.1. Preparation of yeast inoculum

Debaryomyces hansenii P2 previously isolated from naturally fermented sausages (Cano-García et al., 2013) was used as starter in the production of dry fermented sausages. Yeast was cultivated on GPY medium (2% glucose, 0.5% peptone, 0.5% yeast extract, pH 6.0) and the grown cells washed with sterile saline solution (0.9% NaCl) and centrifuged (7000 rpm for 10 minutes at 4ºC) to remove the culture medium. The collected cells were prepared to a concentration of 10^8 c.f.u./ml using dilution plates. The concentrated yeast cells were directly stored at -80°C until their inoculation in dry fermented sausage batches.

2.2. Dry fermented sausages and sampling

Seven batches of dry fermented sausages were manufactured: a control batch (C) was prepared using 70% pork lean meat and 30% pork back fat and 27g/kg NaCl content while six batches were manufactured varying salt and/or pork back fat content with or without yeast inoculation (*Debaryomyces hansenii*). The reformulated batches were: reduced fat (RF); reduced salt (RS); reduced fat and salt (RF+RS); and the same three batches but inoculated with *D. hansenii* yeast (RF+Y, RS+Y, RF+RS+Y). Reduced salt batches were 25 % salt reduced adding 20.25 g/kg NaCl and 6.75 g/Kg KCl. Fat reduced batches were 50 % fat reduced adding 85% lean pork meat and 15% back fat. Appropriate volumes of yeast strain *D. hansenii* P2 suspension were added to the inoculated batches at final concentration of 5 10^6 c.f.u./g of yeast strain. All

113 fermented sausage batches were produced using the following ingredients: lactose (20
114 g/kg); dextrin (20g/Kg); sodium caseinate (20 g/kg); glucose (7 g/kg); sodium ascorbate
115 (0.5 g/kg); sodium nitrite (0.15 g/kg); potassium nitrate (0.15 g/kg) and starter culture
116 (0.1 g/kg) SP318 TEXEL SA-301 (Danisco, Cultor, Madrid, Spain) containing
117 *Lactobacillus sakei*, *Pediococcus pentosaceus*, *Staphylococcus carnosus*. The batches
118 were manufactured under the conditions described by Olivares et al. (2010). The meat
119 mixture was kept at 3-5°C for 24h and then was stuffed into collagen casings of 9.5 cm
120 diameter (FIBRAN, S.A., Girona, Spain) being the final weight of each sausage
121 approximately 700 g. The sausages were subjected to drying in a controlled drying
122 chamber at 10-14°C and 70-85% relativity humidity (RH) for 61 days. The weight
123 losses and pH were measured during ripening to control the drying process.

124 From each batch, 300 g of the meat mixture at 0 days and three sausages at 61
125 days were randomly chosen for microbial and chemical analysis. From each sample,
126 sausage colour was measured, 20 g were taken for microbial analysis and 150 g were
127 minced and used to measure moisture, water activity and pH. The remaining minced
128 sausages from each batch were vacuum packed and frozen at -20°C till subsequent
129 analysis (fat, protein and ions content). In addition, at 61 days the remaining sausages
130 of each batch were vacuum packaged and stored at 4°C for sensory and texture
131 analysis. All the results were expressed as means of the three replicates per 100 g of
132 dry matter at each processing time and batch.

133 **2.3. Microbial analysis**

134 Sausages samples (20 g) were aseptically homogenized with sterile saline
135 solution (1/10) in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 minute and
136 decimal dilutions were prepared. Lactic acid bacteria population was determined by
137 spread plating on MRS Agar anaerobically (Scharlau Chemie SA, Barcelona, Spain)
138 and staphylococci population by using Mannitol Salt Agar (Scharlau Chemie SA,
139 Barcelona, Spain) both medium were incubated at 37°C for 2 days. Yeast count was

140 determined in Rose Bengal Agar with chloramphenicol (RBA) (Conda SA, Madrid,
141 Spain) at 28°C for 3 days.

142 Ten yeast strains isolated from each batch at the initial and final time of the
143 ripening process were subjected to molecular characterization by minisatellite PCR
144 amplification using the M13 primer as described in Cano-García et al (2013). The M13
145 Minisatellite PCR patterns obtained were compared with the originals previously
146 obtained by Cano-García et al (2013).

147 **2.4. Chemical analysis**

148 The measurement of pH, water activity, colour evaluation (CIELab L*, a*, b*),
149 moisture and fat content was performed as described by Olivares et al. (2010).
150 Nitrogen content was determined by the Kjeldhal method and protein was estimated
151 multiplying the nitrogen content by a factor of 6.25.

152 Cations (sodium and potassium) and chloride anion were analyzed by ion
153 chromatography as described by Corral et al. (2013). The concentration of each ion
154 was determined by calibration curves using a set of standard solutions of Na^+, K^+ and
155 Cl^- (Fluka, Switzerland, Sigma, St. Louis, MO). All results were expressed as mg/100g
156 of sample in dry matter.

157 **2.5. Texture profile analysis**

158 Texture profile analysis (TPA) was carried out using TA-XT.plus Texture
159 Analyzer with Texture Exponent software (version 2.0.7.0 Stable Microsystems,
160 Godalming, UK). At the end of the process, three different slices (3.5 cm diameter and
161 1.5 cm thick) of three sausages from each batch were compressed twice to 50 % of
162 their original height as described by Olivares et al. (2010). The main texture
163 parameters (hardness, springiness and cohesiveness) and the secondary parameter
164 chewiness were obtained from the deformation curves.

165 **2.6. Sensory analysis**

166 Sensory analysis of fermented sausages (61 days) was carried out by 81
167 untrained assessors. The analysis was made in a sensory laboratory equipped with

168 individual booths (ISO 8589, 1988). The casing was removed and each sausage was
169 cut into slices of 4mm thickness. One slice of each sausage batch was randomly
170 labelled with three digit codes and presented on a small white plate at room
171 temperature. Water and unsalted bread was provided to clean the palate between
172 samples. A hedonic test was carried out using a 9-hedonic scale labelled on the bottom
173 with “dislike extremely” and on the top “like extremely”. The assessors evaluated their
174 liking of appearance, flavour, taste, hardness, juiciness and overall acceptability. Data
175 acquisition and analysis was performed by Compusense *five* release 5.0 (Compusense
176 Inc., Guelph, ON, Canada).

177 **2.7. Statistical analysis**

178 Analyses of variance (ANOVA) were performed for the chemical and texture
179 parameters to evaluate the differences among samples. A Fisher’s test was used to
180 identify significant ($p<0.5$) differences between types of fermented sausages evaluated.
181 Internal Preference Mapping applied to the mean individual hedonic rates of overall
182 acceptability on all samples was performed. For each product, the coordinates on the
183 preference space determined by the first two components were kept. Then, consumers'
184 hedonic ratings were regressed onto these coordinates, and plotted into the map.
185 Mean values of instrumental parameters were considered as supplementary variables.
186 Clusters analysis was performed to classify consumers according to their preference
187 about dry fermented sausages aroma, taste and overall acceptability. Agglomerative
188 Hierarchical Clustering (AHC) was carried out using Euclidian distance with Ward’s
189 method as the aggregation criterion (XLSTAT 2011 Agglomerative hierarchical
190 clustering). A dissimilarity plot was performed to determine how many clusters were
191 suitable for each analysis. A dendrogram was used to define the cluster structure of the
192 data and support the decision that was made using the dissimilarity plot. All statistical
193 analyses were performed using the statistic software XLSTAT 2011 v5.01 (Addinsoft,
194 Barcelona, Spain)
195

196 **3. Results and discussion**

197 **3.1. Chemical analysis**

198 The pH, a_w and weight losses are shown in table 1. The pH showed a reduction
199 in all batches from an initial value of 5.9-6.1 to 4.9-5.3 considered enough to ensure the
200 safety of meat products together with drying and low a_w values (Papadima et al., 1999).
201 In uninoculated batches, RS and RF+RS showed significantly higher pH values than
202 control batch and RF (Table 1) although this has not been previously reported (Liaros
203 et al., 2009; Olivares et al., 2010; Corral et al. 2013). However, the greatest significant
204 differences were found in yeast inoculated batches as all of them presented the highest
205 significant pH probably due to the ability of yeasts to consume organic acids such as
206 lactic acid (Durá, Flores & Toldrá, 2004).

207 Water activity (a_w) also controlled through the ripening process reached values
208 of 0.90-0.91 thus securing product stability. Few significant differences were observed
209 among batches because the control batch had the lowest a_w (table 1). The effect of salt
210 reduction in a_w values of dry fermented sausages produced different results, no effect
211 was reported in 16 % reduced salt sausages (Corral et al., 2013) while highest a_w
212 values were reported in 50 % reduced salt sausages (Olesen, Meyer & Stahnke, 2004).
213 Moreover fat reduction produced an increase in a_w values (Gómez & Lorenzo, 2013).

214 Weight losses mainly depend on climatic conditions applied for product ripening
215 (Bloukas et al., 1997). The weight losses in all batches were 39.8-41.6 % at the end the
216 process (table 1). The slow ripening conditions applied during process prevented the
217 effect of fat or/and salt reduction on weight loss (Olivares et al. 2010). However, these
218 processing conditions were not able to avoid the differences ($p<0.05$) found when both
219 reductions were applied together in the inoculated *D. hanseni* batch (RF+RS+Y). The
220 highest weight losses produced by fat reduction have been also reported by other
221 authors (Bloukas et al., 1997; Papadima & Bloukas, 1999; Liaros et al., 2009).

222 Table 2 shows the chemical composition of dry fermented sausages. A fat
223 reduction of 10-16% was achieved. The moisture content was the highest ($p<0.05$) in

224 fat reduced batches (Gómez & Lorenzo 2013). However, this effect was not seen
225 ($p>0.05$) in inoculated batches (RF+Y, RS+Y and RF+RS+Y) being in agreement with
226 a_w values obtained. As expected, the highest ($p<0.05$) protein content was found in fat
227 reduced batches due to the highest lean content present as also observed Olivares et
228 al. (2010).

229 A total sodium reduction of 17-20% was achieved (Table 3). Salt reduced
230 batches both uninoculated and yeast inoculated (RS, RF+RS, RS+Y and RF+RS+Y
231 batches) presented significantly lower Na^+ content and higher K^+ content than no salt
232 reduced batches (C, RF, RF+Y) (Table 3). By contrast, no significant differences were
233 found for Cl^- content among batches since, salt reduced batches were substituted by
234 KCl. Overall, fat or/and salt reduction and *D. hanseni* inoculation did not produced
235 significant differences in colour parameters (L*, a* and b*) (Corral et al., 2013) (data
236 not shown).

237 **3.2. Microbial analysis**

238 LAB and staphylococci are essential for the ripening process and play an
239 important role in the safety and organoleptic characteristics of dry fermented sausages
240 (Ravyts, Vuyst & Leroy, 2012). The levels of LAB, staphylococci and yeast population
241 were analyzed at the beginning and end of the ripening process. At the beginning of
242 the process the mean counts of LAB and staphylococci were 10^6 c.f.u./ g d.m. in all
243 batches while for yeasts 10^7 u.f.c/ g d.m. were found in the inoculated batches. At the
244 end of the process, the levels of LAB and staphylococci were within the range of what
245 could be expected in this product (Table 4) (Durá et al., 2004). The population of LAB
246 experienced a growth of 3-4 logarithmic cycles whilst the population of staphylococci
247 was within the same logarithmic units (Andrade, Córdoba, Casado, Córdoba &
248 Rodríguez, 2010). No significant differences were found among batches for LAB level
249 except the RS+Y batch which presented a significant higher LAB level than inoculated
250 C and RF+RS+Y. However, these results were not correlated to the pH values
251 obtained. Concerning staphylococci growth, all batches with fat and/or salt reduction

had lower staphylococci growth than the control batch. Moreover, *D. hanseni* inoculation did not affect the growth of LAB and neither Staphylococcus (table 4). Acidification carried out by LAB causes the inhibition of staphylococci growth (Leroy, Verluyten & De Vuyst, 2006), but in this study the batches which presented the highest pH values were those where the lowest staphylococci counts were found. Therefore, the staphylococci growth could have been affected by fat or/and salt reduction. However, controversial results about their effect on staphylococci have been reported. Several authors reported an absence of effect on staphylococci growth by salt reduction (Corral et al., 2013, Campagnol et al, 2011) while others attributed to KCl the capacity to increase staphylococci growth (Gelabert et al., 2003). With respect to the effect of fat reduction, Del Nobile, Conte, Incoronato, Panza, Sevi & Mariano (2009) reported no effect on staphylococci while Liaros et al. (2009) found a decrease in staphylococci growth in fat reduced sausages attributing it to the lower counts presents in beef and pork meat than in pork back fat. However, Ravyts, Steen, Goemaere, Paelinck, Vuyst & Leroy (2010) reported a limited effect of fat and salt reduction on microbiota growth. In summary, in the present study fat and salt reduction produced a significant decrease on staphylococci growth.

Regarding yeast level at the beginning of the process, all the inoculated batches (RF+Y, RS+Y, RF+RS+Y) showed 10^7 c.f.u./g. yeast while no yeast growth was detected in the uninoculated batches (C, RF, RS, RF+RS). At the end of the process, a low yeast growth was detected in the uninoculated batches (10^4 c.f.u./g) although no differences were detected among batches (Table 4). However, the inoculated batches showed significant higher levels of yeast (10^7 c.f.u./g) than control batches (10^4 c.f.u./g) and this fact was also observed in each respective inoculated versus uninoculated batch.

At the beginning of the process, 100 % of the isolated yeasts from the inoculated batches displayed the original M13 minisatellite pattern of P2 strain indicating the correct inoculation of the batches (Figure 1A Supplementary Material).

280 Similarly, at the end of the process, all isolated yeasts (100%) showed the same
281 pattern as the strain P2 originally inoculated (Figure 1B, Supplementary Material).
282 These results demonstrate that *D. hansenii* P2 was able to survive and replicate in the
283 sausage environment even with fat and/or salt reduction, confirming the dominance of
284 the inoculated P2 *D. hansenii* along the ripening process. In the case of the control
285 batch, solely 20 % of the M13 patterns corresponded to P2 (Figure 1C, Panel C,
286 Supplementary Material). However, the percentage increased between 60% -70% in
287 the uninoculated batches with salt and fat reduction (Figure 1C, Panels RS and RF,
288 Supplementary Material) and reached 90 % in the uninoculated batch with both salt
289 and fat reduction (Figure 1C, Panel RS+RF, Supplementary Material). The presence of
290 *D. hansenii* strains in the uninoculated sausage batches was probably due to the
291 dispersion of the inoculated yeasts along the 61 days of ripening favoured by air
292 circulation in the drying chamber. Nevertheless, the non-inoculated batches presented
293 lower *D. hansenii* P2 counts than the inoculated ones.
294
295 **3.3. Texture profile analysis**
296 TPA parameters were analyzed in the final product (Table 5). Hardness and
297 consequently chewiness were affected by the different formulations whilst no effect
298 was observed on springiness and cohesiveness. In uninoculated batches, RF, RS and
299 RF+RS showed significant higher hardness and chewiness than control batch.
300 Moreover, RS batch showed the highest hardness and chewiness. On the contrary, in
301 inoculated batches, only RF+Y and RF+RS+Y batches showed significant higher
302 hardness than the control batch. The effect of the inoculated *D. hanseni* yeast was
303 significant in salt reduced batches (RS and RS+Y) as it produced a decrease in the
304 hardness and chewiness. However, springiness and cohesiveness were not affected
305 by neither formulation nor *D. hansenii* inoculation. This increase in hardness and
306 chewiness has been already reported in dry fermented sausage when fat was reduced
307 (Olivares et al., 2010). However, salt effect on fermented sausage texture is

contradictory as generally low changes in texture have been reported when KCl was used as unique salt substitute (Gou, Guerrero, Gelabert & Arnau, 1996) or a decrease in sausage hardness (Gimeno et al. 1999); although any reference has indicated an increase in hardness as observed in our uninoculated sausages.

3.4. Sensory analysis

An Internal Preference Mapping was done with mean scores of overall acceptability and the following supplementary parameters which showed significant differences between batches: pH, a_w, weight losses, ions content (Na^+, K^+, Cl^-), fat, protein and moisture content, microbiological analysis (LAB, Staphylococcus and yeast counts), texture analysis (hardness, chewiness) and consumer liking (appearance, aroma and taste) (Figure 1). Two principal components were able to explain the 42.8% of the total variance. PC1 accounted for 22.9% of the variance and distinguished samples according to consumer preferences placing C, RF+RS and inoculated samples on the right quadrants and salt (RS) or fat (RF) reduced samples on the left quadrants. However, PC2 accounted for 19.9% of the variance and distinguished samples by the presence of yeast (in the positive part of the axe are placed samples without yeast inoculation and in the negative part of the axe inoculated samples). Taking into account supplementary parameters plotted, C, RF+RS and RF+Y samples were related with moisture, fat and Na^+ and Cl^- content, staphylococci counts and taste liking; RS and RF samples were related with texture parameters and RS+Y, RF+RS+Y samples were related with pH, protein and potassium content and yeast.

For a better understanding of consumer responses, the preferences for attributes that showed significant differences (taste and overall acceptability) plus the attribute aroma were also analyzed by cluster analysis using Euclidean distances (Figure 2). The attribute aroma was analyzed due to the effect of yeast on sausage aroma (Cano-García et al., 2013). The number of consumers in each cluster was different and depended on the analyzed attribute, thirty-nine and forty-two consumers for aroma, thirty-four and twenty-seven for taste and fifty and thirty-one for overall

acceptability, in cluster 1 and 2 respectively. The sausage preference of each cluster was elucidated by one-way ANOVA. Based on aroma, taste and overall acceptability, cluster 1 preferred fat reduced sausages without yeast inoculation while cluster 2 preferred inoculated and fat reduced sausages (Figure 2).

Salt reduced sausage (RS) was perceived less tasty and the cluster 2 also perceived it less aromatic. Although RS was overall accepted by cluster 2, cluster 1 did not sensory accepted it. Moreover, the yeast inoculation on salt reduced sausages (RS+Y) was accepted better than uninoculated salt reduced sausages by both clusters, even though the sausage aroma and taste did not significantly improve by yeast inoculation. This fact agree with those reported by Corral et al. (2013) and Aaslying et al., (2014) who pointed out that consumers consider reduced sausages an acceptable product although the sensory characteristic are affected. Also, other studies have reported an improvement of sausage aroma and taste using yeast extracts (Campagnol et al., 2011).

Fat reduced sausage was sensory accepted by the two consumer clusters; although, cluster 2 perceived it less tasty and aromatic. However, the yeast inoculation on fat reduction sausage was perceived with more sausage taste and aroma by cluster 2. This result agrees with Olivares et al. (2011) who reported a lower aroma in low fat sausages although Liaros et al. (2009) and Papadima et al. (1999) reported no effect of fat reduction on sausage odour or taste.

When the salt and fat reduction was carried out together, the two clusters perceived this sausage the less tasty than the other formulations, and cluster 1 even perceived it less aromatic. In this case, the yeast inoculation did not improve the sausage taste or aroma.

Nevertheless, different preferences patterns of consumers were found; the yeast inoculation improved the aroma and taste quality when the fat or salt reductions were carried out in dry fermented sausages. Several authors have studied the effect of *D. hansenii* on sausage aroma reporting an increase in some volatile compounds

364 (Andrade et al., 2010) while Olesen & Stahnke (2000) reported few differences
365 between control and yeast inoculated sausages. However, there are not reports about
366 the effect *D. hansenii* inoculation on consumer acceptability and when fat or salt is
367 reduced. In this sense, the addition of yeast extracts produced an increase in the
368 aroma acceptability of sausages (Bolumar, Sanz, Flores, Aristoy, Toldrá & Flores 2006,
369 and Campagnol et al., 2011) while only Flores, Durá, Marco & Toldrá (2004) confirmed
370 the beneficial effect of the inoculation of *D. hansenii* in fermented sausages on
371 consumer aroma acceptability. However, they did not confirm the implantation of the
372 inoculated yeast strain. In addition, further studies are necessary to elucidate the
373 biochemical process involved in the improvement of quality and aroma in low salt and
374 fat dry fermented sausages by the inoculation of *D. hansenii* yeast.
375
376 **4. Conclusion**
377 The inoculation of *D. hansenii* yeast on salt and fat reduced sausages was able
378 to compensate the changes in a_w and texture although it was not able to modify neither
379 the hardness of reduced fat batches nor the decrease in staphylococci growth. In terms
380 of sensory analysis, yeast inoculation improved the aroma and taste quality when fat or
381 salt reductions were done. However, when salt and fat reduction was carried out
382 together, yeast inoculation did not improve sausage taste or aroma. Further studies are
383 necessary to elucidate the biochemical process involved in aroma generation and the
384 interactions with salt and fat reductions.
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386
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393
394
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504

505 **FIGURA LEGENDS**

506

507 **Figure 1.** Loadings of the first two principal components (PC1-PC2) of consumer
508 acceptability (black square), samples (black circles) and instrumental parameters (grey
509 rhombus).

510

511 **Figure 2.** Mean values of the A) aroma; B) taste; C) overall acceptability by consumer
512 cluster. Different letters in each cluster indicate significant differences at $p<0.05$.

513

514 **Figure 1 Supplementary data**. Electrophoretic patterns of minisatellite M13 PCR
515 amplification of strains isolated at 0 d (A) and 61 days (B, C). In each photograph: first
516 lane represents "M" 100 pb ladder (Invitrogen, Carlsbad, CA, USA) followed by the
517 original inoculated P2 yeast (P2) and the 10 isolated strains in the sausage batches.
518 Sausage batches; Control (C), reduced fat (RF), reduced salt (RS), reduced salt and
519 fat (RS+RF) and inoculated reduced fat (RF+Y), inoculated reduced salt (RS+Y) and
520 inoculated reduced salt and fat (RS+RF+Y).

Table captions

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Table 1. Effect of salt and fat reduction on pH, aw and weight losses of dry fermented sausages inoculated with *D. hansenii* yeast.

Table 2. Effect of salt and fat reduction on chemical composition of dry fermented sausages inoculated with *D. hansenii* yeast.

Table 3. Effect of salt and fat reduction on ions content in dry fermented sausages inoculated with *D. hansenii* yeast.

Table 4. Effect of salt and fat reduction on Lactic acid bacteria, Staphylococci and yeast counts in dry fermented sausages inoculated with *D. hansenii* yeast.

Table 5. Effect of salt and fat reduction on texture parameters in dry fermented sausages inoculated with *D. hansenii* yeast.

Table 1. Effect of salt and fat reduction on pH, aw and weight losses of dry fermented sausages inoculated with *D. hansenii* yeast.

	pH		aw		Weight losses (%)	
	Uninoculated	**Inoculated**	**Uninoculated**	**Inoculated**	**Uninoculated**	**Inoculated**
C	5.0 Ab	5.0 Ab	0.906 Ab	0.906 Aa	39.8 Aa	39.8 Ab
RF	4.9 Bb	5.2 Aa	0.914 Ba	0.916 Aa	40.6 Aa	39.8 Ab
RS	5.1 Ba	5.2 Aa	0.912 Aa	0.912 Aa	41.2 Aa	40.6 Ab
RF+RS	5.1 Ba	5.3 Aa	0.916 Aa	0.913 Aa	40.0 Ba	41.6 Aa

Different small letters in the same column indicate significant differences at $P<0.05$.
Different capital letters in each row for each parameter indicate significant differences at $P<0.05$.

Table 2. Effect of salt and fat reduction on chemical composition of dry fermented sausages inoculated with *D. hansenii* yeast.

	Moisture (%)		Fat (%)		Protein (%)	
	Uninoculated	**Inoculated**	**Uninoculated**	**Inoculated**	**Uninoculated**	**Inoculated**
C	46.0 Ac	46.0 Aa	17.5 Aa	17.5 Aa	33.3 Ac	33.3 Ab
RF	46.9 Ab	47.3 Aa	15.3 Abc	15.7 Aab	34.5 Ab	35.4 Aa
RS	46.2 Ac	46.3 Aa	16.7 Aab	16.6 Aa	34.7 Ab	34.9 Aab
RF+RS	47.6 Aa	46.4 Aa	15.0 Ac	14.7 Ab	35.2 Aa	35.9 Aa

Different small letters in the same column indicate significant differences at $P<0.05$.
Different capital letters in each row for each parameter indicate significant differences at $P<0.05$.

Table 3. Effect of salt and fat reduction on ions content in dry fermented sausages inoculated with *D. hansenii* yeast.

	Na^+ (mg/100g d.m.)		K^+ (mg/100g d.m.)		Cl^- (mg/100g d.m.)	
	Uninoculated	**Inoculated**	**Uninoculated**	**Inoculated**	**Uninoculated**	**Inoculated**
C	2925.6 Ab	2925.6 Aa	968.2 Ab	968.2 Ac	4261.8 Aa	4261.8 Aa
RF	3078.1 Aa	3032.7 Aa	1024.8 Ab	1064.7 Ab	4127.2 Aa	4207.4 Aa
RS	2527.5 Ac	2473.6 Ab	1937.6 Aa	1910.7 Aa	4176.7 Aa	4054.9 Aa
RF+RS	2553.9 Ac	2495.0 Ab	1980.9 Aa	1969.0 Aa	4176.8 Aa	4181.3 Aa

Different small letters in the same column indicate significant differences at $P<0.05$.
Different capital letters in each row for each parameter indicate significant differences at $P<0.05$.

Table 4. Effect of salt and fat reduction on Lactic acid bacteria, Staphylococci and yeast counts in dry fermented sausages inoculated with *D. hansenii* yeast.

	LAB (cfu/g dm)		Staphylococci (cfu/g dm)		Yeast (cfu/g dm)	
	Uninoculated	Inoculated	Uninoculated	Inocualted	Uninoculatd	Inoculated
C	2.7E+09 Aa	2.7E+09 Ab	1.7E+07 Aa	1.7E+07 Aa	3.2E+04 Aa	3.2E+04 Ab
RF	2.6E+09 Aa	7.4E+09 Aab	2.3E+06 Ab	1.7E+06 Ab	1.8E+04 Ba	5.5E+07 Aa
RS	3.7E+09 Aa	12.1E+09 Aa	3.1E+06 Ab	1.6E+06 Ab	1.3E+04 Ba	2.7E+07 Aa
RF+RS	2.8E+09 Aa	4.3E+09 Ab	2.0E+06 Ab	1.9E+06 Ab	1.6E+04 Ba	4.1E+07 Aa

Different small letters in the same column indicate significant differences at $P < 0.05$. Different capital letters in each row for each parameter indicate significant differences at $P < 0.05$.

Table 5. Effect of salt and fat reduction on texture parameters in dry fermented sausages inoculated with *D. hansenii* yeast.

	Hardness		Springiness		Cohesiveness		Chewiness	
	Uninoculated	Inoculated	Uninoculatd	Uninoculated	Uniculated	Inoculated	Uninoculated	Inoculated
C	172.7 Ac	172.7 Ac	0.69 Aa	0.69 Aa	0.66 Aa	0.66 Aa	77.4 Ac	77.4 Aa
RF	203.9 Aab	200.7 Aa	0.66 Aa	0.67 Aa	0.65 Aa	0.65 Aa	88.5 Aab	87.3 Aa
RS	217.2 Aa	178.4 Bbc	0.67 Aa	0.71 Aa	0.65 Aa	0.64 Aa	95.4 Aa	80.9 Ba
RF+RS	201.8 Ab	192.7 Aab	0.66 Aa	0.68 Aa	0.65 Aa	0.65 Aa	86.6 Ab	85.2 Aa

Different small letters in the same column indicate significant differences at $p<0.05$. Different capital letters in each row for each parameter indicate significant differences at $p<0.05$.

Figure 1

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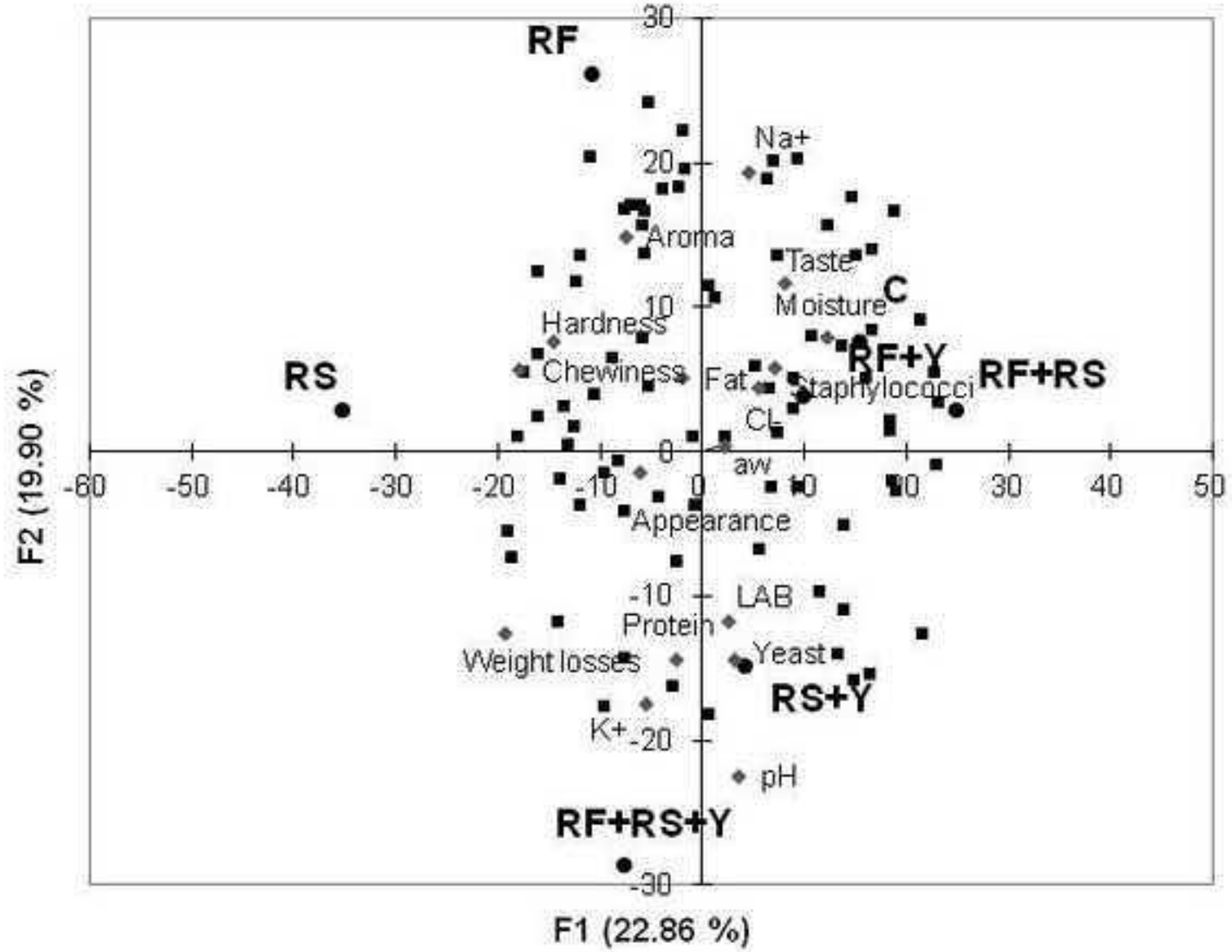

Figure 2

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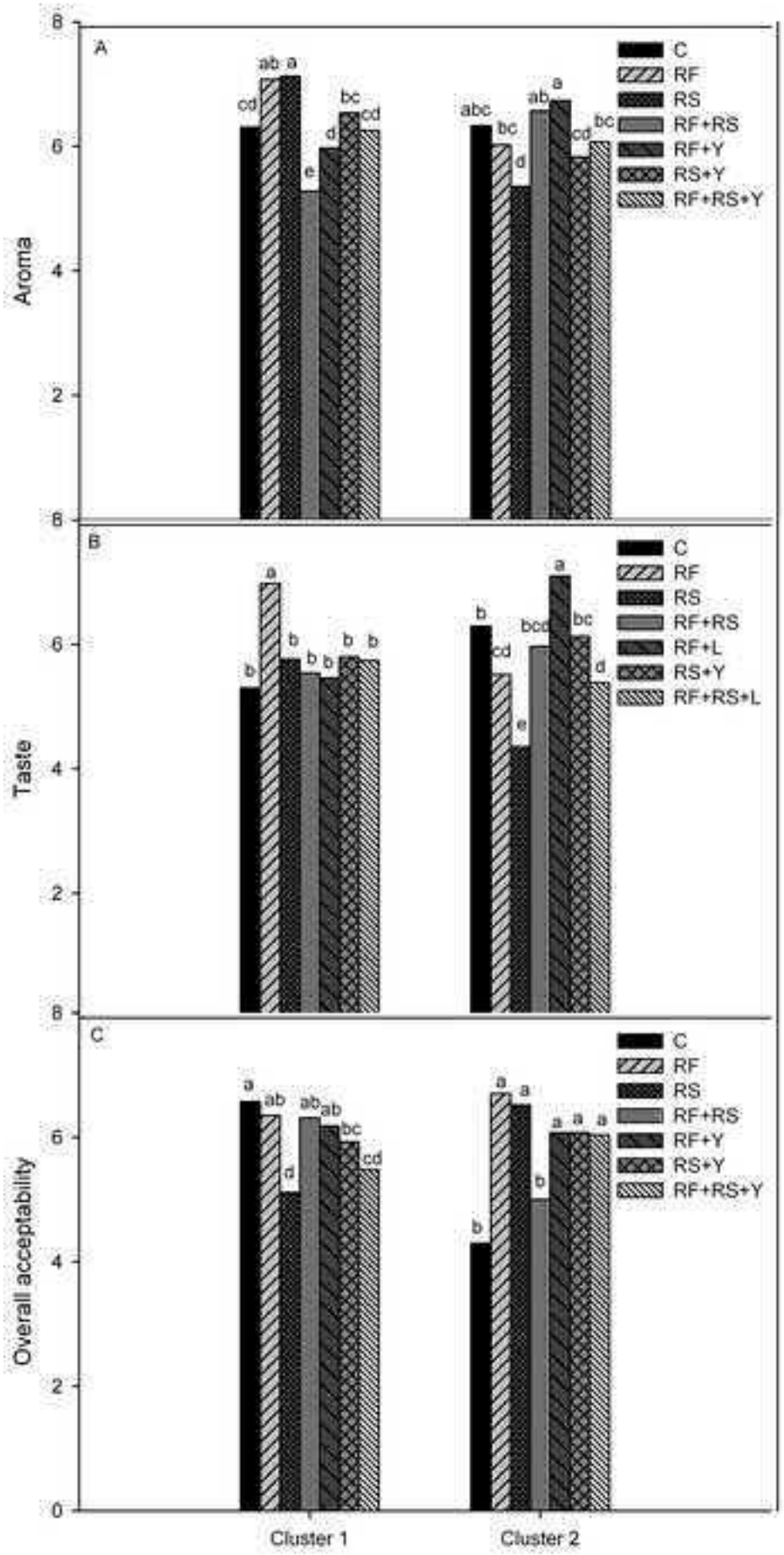
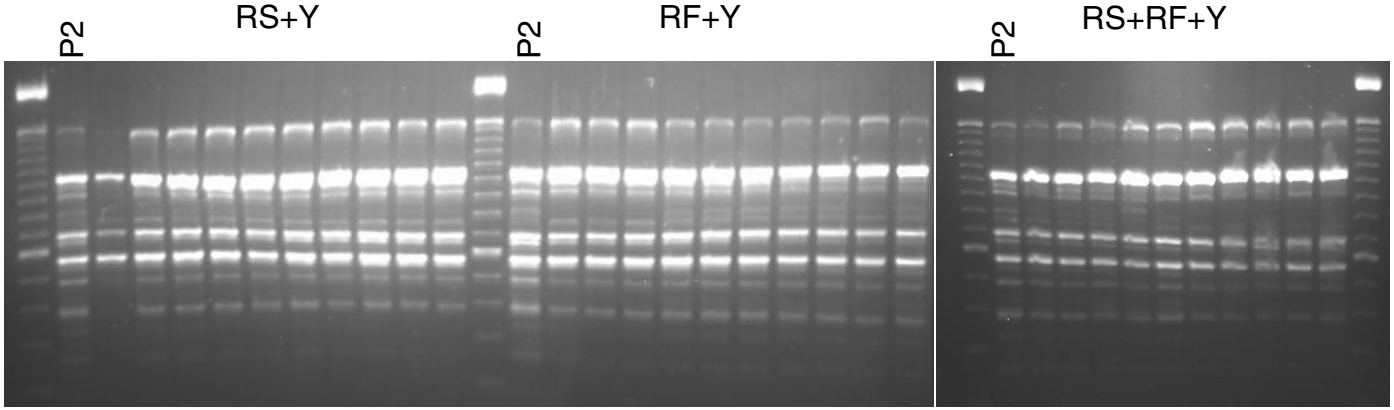
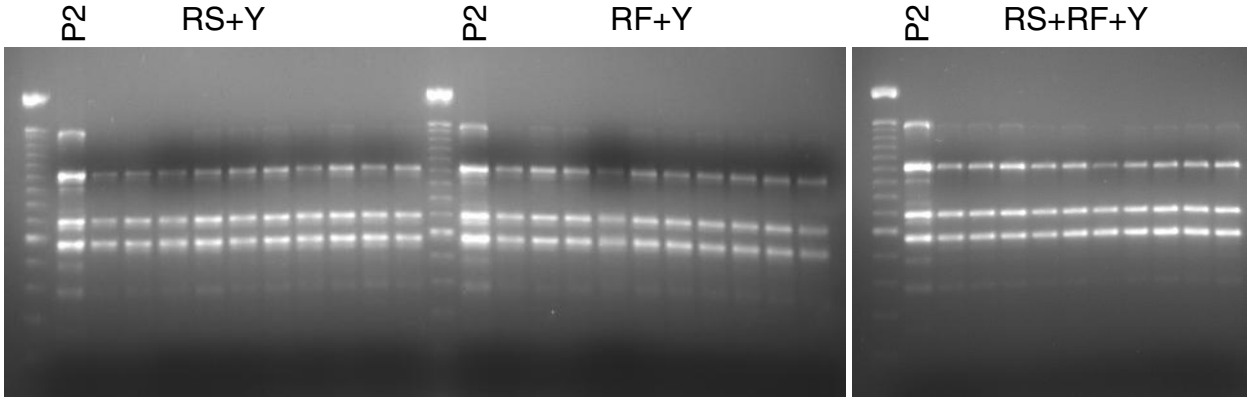


Figure supplementary

A) Time 0 days



B) Time 61 days



C) Time 61 days