Improvement the aroma of reduced fat and salt fermented sausages by *Debaromyces hansenii* inoculation

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Abstract

The effect of *D. hansenii* inoculation in dry fermented sausages with fat and/or salt reductions was studied in terms of lipolysis, lipid oxidation, volatile compounds production and sensory analysis. The aroma of the identified volatile compounds was evaluated by olfactometry analysis while overall aroma perception was evaluated by sensory descriptive profiling. Salt reduction in dry sausages increased lipolysis and contributed to a high oxidation rate and rancid aroma generation while fat reduction resulted in sausages with a high content of aroma compounds from carbohydrate fermentation. The inoculation of *D. hansenii* yeast in the reformulated dry sausages produced an increase in lipolysis and, at the same time, an antioxidant effect. The most important contribution of *D. hansenii* yeast was the increase in aroma compounds derived from amino acid degradation (3-methylbutanoic acid and benzothiazole) and ester activities increasing the perception of fruity and cured aroma notes (2-methylpropanoate, 2-methylbutanoate and 3-methylbutanoate). However, when both reductions were carried out together, *D. hansenii* inoculation did not show a clear effect.

Keywords: fat reduction, salt reduction, *D. hansenii*, volatile compounds, aroma, olfactometry, fermented sausages

1. Introduction

The reduction of salt and saturated fat in meat products is one key point for the meat industry due to dietetic recommendations from the World Health Organization (WHO/FAO, 2003) as they may be associated with cardiovascular diseases. In dry fermented sausages, fat and salt are two essential ingredients in the development of aroma, a crucial sensory attribute. Fat plays an important role in aroma release and generates lipid derived compounds such as aldehydes, ketones, alcohols (Olivares, Navarro & Flores, 2011) while salt controls biochemical and enzymatic key reactions necessary for flavour development in addition to its contribution to the salting out effect (Corral, Salvador & Flores, 2013).

Several authors have studied the effect of fat reduction on volatile compounds and aroma although inconsistent findings have been reported. While, Muguerza, Fista, Ansorena, Astiasarán & Bloukas,et al. (2003) observed a high oxidation level and amount of total volatile compounds in low fat sausages, Olivares et al. (2011) reported a low oxidation and aroma acceptability. On the other hand, low fat sausages are generally perceived too salty due to the relatively large amount of moisture release; therefore, a salt reduction of 20-25 % is recommended in low fat dry sausages (Wirth, 1988). In addition, salt reduction at 16% (Corral et al., 2013) or 25% (Campagnol, Santos, Wagner, Terra & Pollonio, 2011b) reduction and substitution with KCl produced a decrease in aroma perception even though the sausages were acceptable to consumers.

Nevertheless, few studies have been conducted examining the effects of fat plus salt reductions in dry fermented sausages. García-Íñiguez de Ciriano, Berasategi, Navarro-Blasco, Astiasarán & Ansorena (2012) improved the fat profile by using linseed oil (unsaturated fat) and replaced salt by calcium ascorbate, obtaining differences in colour intensity and juiciness. Whilst Beriain, Gómez, Petri, Insausti & Sarriés (2011) used an alginate emulsion and KCl with $CaCl_2$ as fat and salt substitutes, respectively, reporting the lowest score for taste and texture by a trained panel.

Flavour enhancers such as amino acids, glutamate, ribonucleotides, yeast extract and lactate have been used to improve aroma in fat or salt reduced dry sausages (Campagnol, Santos, Morgano, Terra & Pollonio, 2011a and b; Gelabert, Gou, Guerrero & Arnau, 2003; Ruusunen, Simolin & Puolanne, 2001). However, the use of yeast strains can be an alternative as they affect flavour development (Leroy, Verluyten & De Vuyst, 2006). *Debaryomyces hansenii* is resistant to the low a_w and high salt concentration typically found in dry fermented sausages. Its effect on the aroma of fermented sausages has been reported although it depends on the *D. hansenii* strain inoculated (Andrade, Córdoba, Casado, Córdoba & Rodríguez, 2010; Cano-García, Belloch, Flores, 2014a; Olesen & Stahnke, 2000). Moreover, the lipolytic and ester activities reported in *D. hansenii* yeasts (Cano-García et al., 2014a, b) seem to be related to the production of lipid derived and fruity aroma compounds with high aroma impact.

Accordingly, *D. hansenii* inoculation in low fat and salt dry fermented sausages may provide a strategy to improve the aroma lost produced by these reductions. In a previous study, Corral, Salvador, Belloch & Flores (2014a) reported the positive impact on the sensory quality of fat and/or salt reduced dry sausages inoculated with *D. hansenii*, although its biochemical origin was not elucidated. Therefore, the aim of the present work is to evaluate the *D. hansenii* ability to improve aroma in fat and/or salt reduced dry sausages. Moreover, in order to get a better knowledge of the relationship between aroma perception and yeast inoculation, the production of volatile compounds, free fatty acids and lipid oxidation markers were assessed.

2. Materials and methods

2.1. Dry fermented sausages and sampling

Seven batches of dry fermented sausages were manufactured as described Corral et al. (2014a) with a total ripening time of 61 days at 10-14 ºC and 70-85 %. The seven dry fermented sausage batches were: control batch (C); reduced fat (RF);

reduced salt (RS); reduced fat and salt (RF+RS) and the same three reformulated batches but inoculated with the yeast *D. hansenii* (RF+Y, RS+Y and RF+RS+Y). The control batch was manufactured with 70% pork lean meat and 30% pork back fat and 27g/kg NaCl content while 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 (Cano-García et al., 2014a) were added to the inoculated batches at final concentration of 5 x 10^6 c.f.u./g of meat batter.

From each batch, 300 g of the meat mixture at 0 days and three sausages at 61 days were randomly collected from each batch. The sausage sample was minced, vacuum packed and frozen at -20ºC for moisture, lipid, free fatty acids and TBARS analyses. At 61 days, three sausages per batch were taken wrapped in aluminum foil, vacuum packed and stored at -80ºC for volatile and aroma analysis. In addition, three additional sausages were directly used for sensory analyses. All the results were expressed as the mean of three replicates per batch.

2.2. Lipolysis and lipid autooxidation analysis

Lipolysis was determined by analysis of free fatty acids (FFA). Total lipids were extracted from 5 g of minced sausages as described by Corral et al. (2013). FFA were determined in total lipids and methylated as described by Olivares et al. (2011) using heneicosanoic acid (C21:0) as internal standard.

The analysis was performed with an Agilent HP 7890A gas chromatograph (GC) equipped with a flame ionisation detector (FID) set at 240°C and autosampler (Agilent Technologies 7683B). 1 µl was injected in split injector (split ratio 100:1) set at 220°C.The fatty acid methyl esters were separated in HP-88 capillary column (Agilent, Las Rozas, Spain, 100m, 0.25 mm i.d, 0.25µm film thickness) using helium at a flow rate of 26.03 cm/s. The oven temperature began at 140ºC for 10 min, ramped to 190ºC at 4ºC/min, held at 190ºC for 15 min, ramped to 220ºC at 2ºC/min and held for 10 min. FAMEs were identified by comparing their retention times with those of standard fatty

131 acid methyl esters. For quantification, response factors of the standards respect to the
132 internal standard (C21:0) were used. The results were expressed as mg of fatty
133 acid/100g of dry fermented sausages in dry matter. Moisture content was determined
134 as described by Corral et al (2014a).

135 Lipid oxidation was determined by the thiobarbituric acid reactive substances
136 (TBARS) method as described by Corral et al. (2013). Results were expressed as mg
137 malonaldehyde (MDA)/kg in dry matter.

138 **2.3. Extraction of volatile compounds**

139 Extraction of headspace (HS) volatiles compounds was performed using solid
140 phase microextraction (SPME) with an 85 µm Carboxen/ Polydimethylsiloxane
141 (CAR/PDMS) fibre as described by Corral et al. (2013). Five grams of minced sausage
142 were weighted into a 20 ml HS vial sealed with a PTFE faced silicone septum and 0.75
143 mg of BHT was added. Before extraction, the vial was equilibrated at 37 ºC for 30 min
144 and then, SPME fibre was exposed to the headspace during 3h at 37 ºC.

145 **2.4. Gas chromatography-mass spectrometry (GC-MS)**

146 The identification and quantification of HS volatile compounds was performed
147 using an Agilent HP 7890 series II GC (Hewlett- Packard, Palo Alto, CA) with an HP
148 5975C mass selective detector (Hewlett-Packard) equipped with Gerstel MPS2
149 multipurpose sampler (Gerstel, Germany). Extraction of HS volatile compounds was
150 performed using SPME as indicated above.

151 The compounds extracted by the fibre were desorbed in the injection port of the
152 GC-MS for 5 min at 240 ºC with purge valve off (splitless). The analysis of volatile
153 compounds in the GC-MS was performed as described Olivares et al. (2011). The
154 compounds were identified by comparison with mass spectra from the library database
155 (Nist'05), kovats retention index (Kovats, 1965) and by comparison with authentic
156 standards. The quantification of volatile compounds was done in SCAN mode using
157 either total or extracted ion chromatogram (TIC or EIC) on an arbitrary scale. The
158 results were expressed as abundance units (AU) 10^{-6}.

159 **2.5. Gas-chromatrography-olfactometry**

160 The analysis of aroma compounds extracted by SPME was performed using a
161 gas chromatograph (Agilent 6890, USA) equipped with a FID and sniffing port
162 detectors (ODP3, Gerstel, Mülheim an der Ruhr, Germany) as described by Olivares et
163 al. (2011). Each assessment was carried out according to Olivares et al. (2011). Four
164 trained panellists evaluated the odours from the GC-effluent of the sausages (61 days).
165 The detection of an odour by less than three assessors was considered to be noise.

166 Compounds were identified using the following techniques: comparison with
167 mass spectra, comparison with kovats retention indices of authentic standards injected
168 in the GC-MS and GC-O, and by coincidence of the assessors's descriptors with those
169 in the Fenaroli's handbook of flavour ingredients (Burdock, 2002).

170 **2.6. Sensory analysis**

171 An Aroma profile analysis was performed by a panel of 8 trained judges with
172 previous experience in quantitative descriptive analysis (QDA). The training of the
173 panel was described previously (Corral et al., 2014b). The selected aroma descriptor
174 during training were pepper, rancid, sour, cheesy, fruity, cured and stable and they
175 were scored using a 10-cm intensity scale (1= no perceived; 10=very intense).

176 The evaluation of dry fermented sausage aroma was done using fresh sliced
177 sausages (4 mm thickness). The sample evaluation sessions were carried out in
178 duplicate with a balanced complete block experimental design (Williams design for 7
179 samples and 8 assessors) using Compusense five release 5.0 (Compusense Inc.,
180 Guelph, Ontario, Canada). Each assessor evaluated the aroma intensity of the
181 selected aroma descriptors at individual booths in a standardized test room (ISO 8589).
182 Samples were presented in petri dish coded with a random three digit numbers. To
183 avoid aroma carryover, coffee beans were provided inside a vial to allow assessors to
184 smell them between samples.

185 **2.7. Statistical analysis**

186 Analyses of variance (ANOVA) were performed for FFAs, TBARS and volatile
187 compounds to elucidate the differences among samples. Differences between
188 particular sample means were analysed according to Fisher's least significant
189 difference (LSD) test. To check panel performance for each aroma descriptor, a two
190 factor analysis of variance (ANOVA) was done (assessors and samples and their
191 interaction as factors). In addition, principal component analysis (PCA) was performed
192 to evaluate the relationships among aroma descriptors and different parameters (FFAs,
193 TBARS and volatile compounds) among sausages. The sensory aroma intensities
194 were used as parameters and aroma compounds abundance, FFA and TBARS value
195 as supplementary variables. All statistical analyses were performed using the statistic
196 software XLSTAT 2011) (v5.01) (Addinsolft, Barcelona, Spain).
197
198 **3. Results**
199 The chemical composition and microbiology analysis of the seven sausage
200 batches previously reported (Corral et al., 2014a) confirmed the yeast inoculation and
201 fat (10-16%) and salt (17-20%) reduction in the manufactured batches. Presence of the
202 yeast *D. hansenii* was checked by microbial and molecular analyses and the
203 dominance of the yeast inoculated in the sausages was confirmed (Corral et al. 2014a).
204 **3.1. Lipolysis and lipid oxidation**
205 Table 1 shows the levels of FFA at the end of the ripening process. Initially, no
206 differences in total FFA concentration were observed among batches and levels of
207 144-191 mg/100g dm were detected (data not shown). During the ripening process,
208 FFA concentrations increased in all batches to around 2000 mg/100g dm. MUFA
209 showed a greater increase than PUFA and SFA, in particular C18:1.
210 At the end of processing, no significant differences were detected in total FFA
211 concentration in uninoculated batches. The fat reduction scarcely showed significant
212 differences for SFA, MUFA and PUFA. Only C18:0 concentration was significantly
213 higher in the fat reduced batches than in C batch. On the other hand, salt reduced

batch (RS) had a higher ($p<0.05$) SFA level than control batch (C) as also was observed in fat and salt reduced batch (RF+RS). In inoculated batches, RS+Y and RF+Y, a higher release of total FFA than in the C batch was observed mainly due to the increase in SFA and MUFA. However, this effect was not detected when both reductions (RS+RF+Y) were carried out together. In summary, the effect of *D. hansenii* yeast inoculation produced a significant increase of the lipolysis in RF+Y batch in contrast to the FFA detected in the RF batch.

Regarding lipid oxidation, TBARS values increased throughout the ripening process from an initial value of 0.4 to 1.7-2.5 mg MDA/kg dm (Table 1). No differences in TBARS values among batches were observed at the beginning of process (data not shown). At the end of the process, salt reduced batches (RS, RF+RS) showed the highest ($p<0.05$) lipid oxidation values. In contrast, the presence of the yeast produced a decrease of the TBARS values in RS+Y and RF+Y batches in comparison to their respective uninoculated batches although it was only significant in RF+Y batch.

3.2. Volatile compounds

The SPME headspace composition cannot be compared with other studies in which other SPME fibres or other extraction techniques have been used as it depends on many factors (fibre, extraction conditions, etc.). However, the present extraction technique allows determination of the effect of the studied factors (fat and salt reduction and yeast inoculation) on the volatiles of the sausages.

A total of 95 volatiles compounds were identified in the sausage headspace; although two of them were tentatively identified (Table 2). The identified volatile compounds were classified according to the most likely origin (Ordóñez, Hierro, Bruna & De La Hoz, 1999): from lipid autooxidation reactions (27), or from bacterial metabolism by lipid β-oxidation (7), carbohydrate fermentation (9), amino acid degradation (29) and esterase activity (15) reactions and, finally, a group of unknown or contaminant compounds (8) (Table 2). The most abundant volatile compounds were

241 those originated from carbohydrate fermentation and amino acid degradation reactions
242 which represented 15-55 % and 11-43% of the total extracted area, respectively.

243 In general, volatile compounds originated from lipid autooxidation reactions
244 were mainly affected by salt reduction and *D. hansenii* inoculation (Table 2). Among
245 the uninoculated batches, salt reduced batch (RS) showed the highest HS abundance
246 of the main lipid autoxidation volatile compounds, especially the significant increase of
247 hexanal. Other linear aldehydes such as pentanal and nonanal also showed a highest
248 HS abundance. On the other hand, fat reduction affected few lipid autooxidation volatile
249 compounds. Only 1-propanol, octanoic and decanoic acid showed a higher HS
250 abundance in the RF batch while propanoic acid had a lower HS abundance than in the
251 C batch. When both reductions were carried out together, several volatile compounds
252 were also generated in higher abundance than in the C batch. Among inoculated
253 batches, a significant increase in some volatile compounds was observed in all batches,
254 such as propanal, 1-propanol, 2-methylfuran, 2-pentylfuran, hexanoic acid, 2-ethyl-1-
255 hexanol and decanoic acid. However, the effect of *D. hansenii* inoculation was
256 significant in the RS+Y batch as it partly inhibited the lipid oxidation by decreasing the
257 concentration of many of the aldehydes observed in the RS batch. In addition, the
258 reduction observed in hexanal abundance was significant.

259 Volatile compounds coming from lipid β-oxidation were also affected by salt
260 reduction and *D. hansenii* inoculation in salt reduced batches. Salt reduced
261 uninoculated batches (RS and RF+RS) were characterized by a high abundance of 2-
262 nonanone, 1-octen-3-ol, 3-pentanone and 2-undecanone. The effect of *D. hansenii* was
263 significant in the RS+Y batch as reduced the abundance of 3-pentanone, 4-heptanone,
264 2-heptanone and 1-octen-3-ol indicating a decrease in the lipid β-oxidation reactions.

265 Regarding the volatiles originated from carbohydrate fermentation reactions,
266 they were affected by the different formulations. Uninoculated salt reduced batches
267 (RS and RF+RS) had the lowest abundance of acetic acid, 3-hydroxy-2-butanone, 2,3-
268 butanediol and butanoic acid (Table 2). In contrast, the RF batch showed the highest

abundance of acetic acid, 2,3-butanediol and butanoic acid. The effect of the inoculated *D. hansenii* was significant as it produced the highest ethanol and acetaldehyde abundance whilst a significant reduction of 2,3-butanedione, acetic acid, 3-hydroxy-2-butanone, 2,3-butanediol and butanoic acid was observed in all inoculated batches.

With respect to volatile compounds originated from amino acid degradation reactions, the most abundant compound was 3-methyl-1-butanol. In general, these compounds were mainly affected by fat reduction and *D. hansenii* inoculation (Table 2). Uninoculated fat reduced batches (RF and RF+RS) were characterized by the highest abundance of branched alcohols (2 and 3-methyl-1-butanol), in addition to a high abundance of branched acids (2-methylpropanoic and 2- and 3-methylbutanoic acids) and sulphur compounds (3-thiophenethiol, 3-methylthiopropanol, and benzothizole) in RF+RS batch. However, salt reduction (RS batch) produced a decrease in the abundance of several compounds (2-methyl-3-buten-2-ol, 2-methyl-1-propanol, 3-methyl-3-buten-1-ol, 3-methyl-1-butanol, methylpyrazine, 2,6-dimethylpyrazine and benzyl alcohol) while other compounds were increased (2-acetyl-1-pyrroline, 3-methlythiopropanal, benzaldehyde and benzene acetaldehyde). The effect of *D. hansenii* was significant as observed by the highest HS abundance of methyl branched aldehydes, acids and sulphur compounds in all inoculated batches.

Ester compounds originated from microbial activity were affected by reformulation and *D. hansenii* inoculation. When fat and salt reductions were carried out together (RF+RS) the highest significant abundance of many ester compounds was observed, while salt reduction (RS) only increased ethyl hexanoate and fat reduction (RF) augmented ethyl acetate, butyl acetate and ethyl octanoate abundance (Table 2). In contrast, inoculation of *D. hansenii* produced a significant increase of many ethyl ester compounds in all the inoculated batches being characterized by the high increase of ethyl 2-hydroxypropanoate and in lowest proportion of ethyl 2-methyl and 3-methylbutanoate.

297 Several compounds from unknown or contaminant origin were identified, being
298 the most abundant carbon disulphide. While the highest abundance of this compound
299 was observed in the RF+RS batch, no effect due to yeast inoculation was detected.
300 An olfactometry analysis performed to determine which volatile compounds
301 contributed to sausage aroma detected twenty-seven aroma active zones although five
302 of them were not identified (Table 3). In addition to the green notes produced by lipid
303 oxidation derived compounds, it was important the contribution of compounds derived
304 from amino acid degradation and ester activity that contributed to toasted-savoury and
305 fruity notes respectively. The abundance of these aroma compounds in the dry
306 sausage batches was represented in figure 1. The reformulation produced a significant
307 effect. Salt reduction (RS) produced the highest abundance of aroma compounds
308 derived from lipid autooxidation and β-oxidation and the lowest from carbohydrate
309 fermentation reactions. Fat reduction (RF) increased the aroma compounds derived
310 from carbohydrate fermentation reactions. In contrast, when both reductions (RF+RS)
311 were carried out together, an increase in all the aroma compounds from the different
312 origins was observed except from carbohydrate fermentation and unknown origins. The
313 effect of *D. hansenii* was significant as produced the highest abundance of aroma
314 compounds derived from amino acid degradation, ester activity and unknown
315 compounds and the lowest of carbohydrate fermentation aroma compounds (Figure 1).
316 In addition, yeast inoculation reduced significantly the aroma compounds derived from
317 lipid autooxidation and β-oxidation reactions in the salt reduced batch (RS+Y) that were
318 highly increased in the uninoculated batch (RS).
319
320 **3.3. Sensory analysis**
321 In order to study the effect of reformulation and yeast inoculation on the
322 perception of sausage aroma an aroma profile analysis was carried out (figure 2). The
323 effect of yeast inoculation was compared against the control sausage for each
324 reformulation; RF (figure 2A), RS (figures 2B) and RF+RS (figure 2C). Fat reduction did

not affect the aroma perception but the inoculation of *D. hansenii* significantly increased the perception of fruity and cured aromas (Figure 2A). Moreover, salt reduction did not affect the aroma perception but the inoculation of *D. hansenii* significantly increased the perception of stable and cured aromas and although rancid aroma was increased it was not significant (Figure 2B). Finally, when both reductions were done together a higher intensity of cured and fruity aroma was perceived in inoculated and uninoculated batches than in the control sausages (Figure 2C).

4. Discussion

The reformulation of fermented sausages in terms of fat and salt reduction produced important changes in aroma compounds and aroma perception. First, salt reduction produced an increment in lipolysis, TBARS and aroma compounds derived from lipid autooxidation and β-oxidation reactions (Corral et al., 2013). This fact indicated that lipolysis was affected by salt content (Molly, Demeyer, Civera & Verplaaetse 1996; Toldrá, 1992) whereas the highest generation of SFA and MUFA pointed out an activation of the lipase activities in the salt reduced sausages (Stahnke, 1995). An opposite effect, activation of muscle acid lipase activity by salt, had been previously reported (Motilva & Toldrá, 1993). In addition to this, a positive correlation between the lipid oxidation value (TBARS) and hexanal abundance in salt reduced sausages was found ($r = 0.82$) (Olivares et al. 2011). Therefore, salt reduction in dry sausages increased lipolysis and contributed to a high oxidation rate and rancid aroma generation.

On the other hand, the small fat reduction achieved (10-16%) had a limited impact on lypolisis, TBARS values and aroma compounds derived from lipid oxidation reactions. Even though an increase in aroma compounds derived from carbohydrate fermentation was detected (Olivares et al., 2011). In contrast, when salt and fat reductions were carried out together not only lipolysis and lipid oxidation values were increased but also many of the aroma compounds. The highest abundance of volatile

353 compounds coming from amino acid degradation reactions could be related to the
354 largest proportion of lean meat in these sausages (Olivares et al., 2011). In addition,
355 the highest abundance of ester compounds could be due to the interaction of the fat
356 and salt reduction effects, on microbial activity. Previously, a weak effect of fat or salt
357 content on sausage microbiota was reported (Ravyts, Steen, Goemaere, Paelinck, De
358 Vuyst & Leroy, 2010) probably due to the limitation of the analysis performed using
359 static headspace that did not allowed the detection of ester compounds in the
360 sausages.

361 In relation to the effect of *D. hansenii* on dry sausages, a significant increase in
362 lipolysis was detected (Table 1) (Bolumar, Sanz, Flores, Aristoy, Toldrá & Flores, 2006)
363 which can be related to the lipolytic activity found in *D. hansenii* (Cano-García et al.,
364 2014b). In contrast, the antioxidant effect produced by *D. hansenii* in salt reduced and
365 partially in fat reduced sausages, can be due to the decomposition of hydrogen
366 peroxide by the catalase activity of *D. hansenii* (Segal-Kischinevzky, Rodarte-Murguía,
367 Valdés-López, Mendoza-Hernández, González & Alba-Lois., 2011). This antioxidant
368 effect was also confirmed in dry sausages (Cano-García et al. 2014a, Flores, Durá,
369 Marco & Toldrá 2004) although not observed by Andrade et al. (2010). In addition, the
370 inhibition of *Staphylococci* growth by *D. hansenii* (Corral et al., 2014a; Durá, Flores &
371 Toldrá, 2004a) resulted in a decrease in the abundance of compounds from the β-
372 oxidation reactions as they are responsible for their generation. On the other hand, the
373 observed decrease in aroma compounds derived from carbohydrate fermentation in
374 yeast inoculated sausages was related to the lowest abundance of acetic acid although
375 opposite results had been found by Cano-García et al. (2014a).

376 The most notable effect of *D. hansenii* was observed in the generation of
377 volatile compounds derived from amino acid degradation and ester activities. In
378 inoculated sausages, a clear catabolism of branched amino acids yielded α-keto acids
379 (2-methylpropanoic, 2 and 3-methlbutanoic) which were descarboxylated to branched
380 aldehydes (2-methylpropanal, 2 and 3-methylbutanal, 3-methyl-2-butenal) and reduced

381 to corresponding branched alcohol (2-methyl-1-propanol, 2 and 3-methyl-1-butanol and
382 3-methyl-2-buten-1-ol) by means of Ehrlich pathway (Durá, Flores & Toldrá, 2004b).
383 This fact agrees with other authors studies (Andrade et al., 2010; Flores et al., 2004;
384 Bolumar et al., 2006) although it was not clearly appreciated in a previous study (Cano-
385 García et al., 2014a). Moreover, sulphur compounds, derived from amino acids
386 degradation reactions, were detected in a high proportion in the yeast inoculated
387 sausages contributing actively to sausage aroma due to their low odour thresholds
388 (Mottram, 1998). The ability of *D. hansenii* to produce volatile sulphur compounds has
389 been previously reported (Olesen & Stahnke, 2000, Cano-García et al. 2014b)
390 although it depends on media composition and on the other starter cultures present
391 (Cano-García et al., 2014a). The inoculated *D. hansenii* also enhanced the production
392 of ester compounds in dry sausages (Flores et al. 2004; Bolumar et al., 2006; Cano-
393 García et al., 2014a; Andrade et al., 2010) by means of the esterification of carboxylic
394 acids and alcohols such as ethanol which was found in the highest abundance.

395 In order to understand the interrelationships between aroma perception and
396 the chemical parameters analyzed (aroma active compounds, FFA and oxidation index
397 TBARS) in the dry sausages, a principal component analysis was performed (figure 3).
398 The PCA biplot described 75.3% of the variability by the two first principal components.
399 PC1 is the most important variable in terms of differences among sausages because it
400 accounts for 51.3% of the total variability while PC2 accounts for 24.0% of the
401 variability. PC1 was strongly related to yeast inoculation placing the inoculated batches
402 on the positive part of PC1. While PC2 distinguished the samples according to fat or
403 salt reduction. The distribution of the variables according to PC1 suggested that fruity,
404 cured and sour aromas of inoculated batches were associated to aroma compounds
405 such as ethyl esters, acids, sulphur and nitrogen compounds which produced fruity and
406 savoury odour notes in olfactometry (GC-O) analysis (table 3). PC2 was related to
407 rancid, cheesy and stable aromas of salt reduced batches mainly dominated by FFA
408 concentration (MUFA and PUFA) and hexanal abundance. These results confirm not

409 only the negative effect of salt reduction on aroma generation and perception and the
410 contribution of specific aroma compounds but also the aroma enhancement produced
411 by *D. hansenii* P2 yeast. Moreover, it is essential to select the appropriate yeast strain
412 for aroma enhancement as wide differences among yeast strains in ester and sulphur
413 production had been detected (Cano-García et al., 2014a, b). Nevertheless, the limited
414 impact of *D. hansenii* on sausage sensory characteristics reported by other authors
415 (Cano-García et al., 2014a; Olesen & Stahnke, 2000) may be due to the metabolic
416 activity of these yeasts as it is affected by many factors such as; meat ingredients,
417 technological parameters and also other microorganism present throughout the
418 ripening process (Ravyts, De Vuyst, L. & Leroy, 2012) which affect the final sensory
419 characteristics.
420
421 **5. Conclusion**
422 In summary, the reformulation of sausages by the decrease in fat and salt
423 content produced important changes in the generation of aroma compounds and
424 aroma perception but the inoculation of *D. hansenii* was shown to compensate for
425 these effects and improve the sensory characteristics of the dry sausages. The most
426 important contribution of *D. hansenii*, in addition to the lipolysis increase and
427 antioxidant effect, was the enhance in aroma compounds derived from amino acid
428 degradation and ester activity increasing the perception of fruity and cured aroma notes.
429 However, when both reductions were carried out together, *D. hansenii* inoculation did
430 not show a clear effect.
431
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437

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545 **FIGURE LEGENDS**

546

547 **Figure 1.** Effect of fat and salt reduction on total aroma compounds (expressed as total
548 ion current abundance units TIC AU) of dry fermented sausages (61 days) inoculated
549 with *D. hansenii*. Aroma compounds derived from: A) lipid autooxidation; B) Lipid β-
550 oxidation; C) Carbohydrate fermentation; D) Amino acid degradation; E) Esterase
551 activity; F) Unknown or contaminant compounds. Different letters in the same group
552 (uninoculated and inoculated) indicate significant differences ($p<0.05$) among batches.
553 Different number superscripts indicate significant differences ($p<0.05$) between
554 uninoculated and inoculated samples within the same reformulation.

555

556 **Figure 2.** Aroma profile analysis of control sausage (solid line) compared to: A) Fat
557 reduced sausages (RF and RF+Y), B) Salt reduced sausages (RS and RS+Y), C) Fat
558 plus salt reduced sausages (RF+RS and RF+RS+Y). Asterisks in each aroma
559 descriptor indicate significant differences at $p<0.05$.

560

561 **Figure 3.** Loadings of the first two principal components (PC1-PC2) of trained panel,
562 samples (●), aroma descriptors (▲) and aroma active compounds, lipolysis and lipid
563 oxidation (■).

Table 1. Effect of fat and salt reduction on free fatty acid content (FFA, mg/100g dm) and TBARS value (mg MDA/kg dm) of dry fermented sausages (61days) inoculated with *D.hansenii* yeast.

	Uninoculated									Inoculated									
Free fatty acids	**C**		**RF**		**RS**		**RF+RS**		**SEM**	**C**		**RF+Y**		**RS+Y**		**RF+RS+Y**		**SEM**
C12:0	2.3		2.2	^{2}	2.4		2.7		0.2	2.3		2.6	$^{1.0}$	2.8		2.5		0.1
C14:0	20.4		20.9	^{2}	22.5		22.2		1.9	20.4	b	23.9	ab^{1}	25.9	a	21.1	b	1.2
C15:0	1.6		1.6	^{2}	1.7		1.7		0.1	1.6	c	1.8	ab^{1}	2.0	a	1.7	bc	0.1
C16:0	310.5	c	358.7	bc^{2}	398.3	ab	420.2	a	14.6	310.5	c	395.3	b^{1}	448.6	a	350.2	bc	15.9
C17:0	5.9		6.6		6.6		6.4		0.5	5.9	b	7.0	ab	7.9	a	6.6	b	0.4
C18:0	138.7	c	161.5	b^{2}	181.0	ab	186.1	a	6.8	138.7	d	180.5	b^{1}	200.4	a	162.9	c	6.4
C20:0	1.2		1.2	^{2}	1.2		1.3		0.1	1.2		1.3	$^{1.0}$	1.4		1.2		0.1
SFA	480.6	c	552.8	bc^{2}	613.7	ab	640.6	a	22.9	480.6	c	612.5	b^{1}	689.1	a	546.1	bc	23.1
C14:1	0.7		0.7		0.7		0.7		0.1	0.7		0.7		0.8		0.7		0.1
C16:1	47.0		49.9	^{2}	48.3		47.0		3.9	47.0	bc	55.2	ab^{1}	60.0	a	43.9	c	3.2
C17:1	11.3		13.6		12.3		14.2		2.1	11.3		12.2		12.4		13.3		2.3
C18:1	701.9		704.0	^{2}	776.2		791.5		37.5	701.9	b	789.5	ab^{1}	898.7	a	670.1	b	36.3
C20:1 n9	19.9		19.8		21.3		21.1		1.7	19.9		21.7		22.5		18.4		1.1
MUFA	780.1		788.0	^{2}	858.2		873.4		45.1	780.1	b	879.3	a^{1}	993.3	a	745.7	b	45.0
C18:2n6	455.2		434.2		478.6		499.6	^{1}	17.6	455.2	b	451.2	b^{1}	507.3	a	410.6	b^{2}	12.5
C18:3 n3	18.3		18.0		19.0		18.6		1.5	18.3		20.0		21.1		17.8		1.1
C18:3 n6	0.6		0.6		0.7		0.6		0.1	0.6		0.7		0.7		0.6		0.0
C20:2 n6	18.1		16.8		18.4		17.5		1.4	18.1		18.0		19.9		16.1		0.9
C20:3 n6	2.8		3.0	^{2}	3.0		3.4		0.2	2.8		3.3	$^{1.0}$	3.4		3.1		0.2
C20:4 n6	51.0	b	55.9	b^{2}	52.8	b	69.5	a	3.4	51.0		61.9	$^{1.0}$	63.1		58.9		3.7
C22:4 n6	8.9		10.1	^{2}	10.2		11.7		0.8	8.9	b	11.2	a^{1}	11.6	a	11.2	a	0.7
C20:5 n3	5.3		5.1	^{2}	4.7		5.6		0.4	5.3		5.6	$^{1.0}$	5.1		4.9		0.4
C22:5 n3	18.7		19.8	^{2}	18.7		21.1		1.6	18.7		21.4	$^{1.0}$	22.4		19.0		1.1
C22:6 n3	1.8		2.0		1.9		1.9		0.2	1.8	b	2.1	ab	2.4	a	1.7	b	0.1
PUFA	580.5		565.5		608.9		649.3	^{1}	35.0	580.5		595.2		657.3		543.7	$^{2.0}$	29.8
TOTAL FFA	1775.8		1885.6	^{2}	1963.7		1951.1		151.3	1775.8	b	2087.0	ab^{1}	2198.8	a	1829.1	b	106.0
TBARS	1.7	b	1.7	b^{1}	2.0	ab	2.5	a	0.2	1.7	b	0.9	c^{2}	1.7	b	2.4	a	0.2

Different letters in the same row of each group (uninoculated and inoculated) indicate significant differences at $p<0.05$ among batches. Different number superscript indicate significant differences at $p<0.05$ between uninoculated and inoculated samples within the same reformulation.

Table 2. Effect of fat and salt reduction on volatile compounds (expressed as AU x 10^{-6}) of dry fermented sausages (61 days) inoculated with *D.hasenii* yeast.

		Uninoculated									Inoculated									
Compound	**LRI[A]**	**C**		**RF**		**RS**		**RF+RS**		**SEM**	**C**		**RF+Y**		**RS+Y**		**RF+RS+Y**		**SEM**	
Lipid autooxidation		**96.24**	**b[B]**	**118.49**	**b**	**1420.93**	**a[1]**	**246.74**	**b**	85.59	**96.24**	**c**	**112.19**	**c**	**229.10**	**b[2]**	**278.55**	**a**	18.39	
Propanal	523	0.78	b	0.83	b[2]	7.74	a[1]	1.36	b[2]	0.28	0.78	b	2.28	a[1]	3.08	a[2]	2.42	a[1]	0.26	
1-Propanol	611	2.01	c	4.99	b	6.77	a	4.49	b	0.48	2.01	b	9.74	a	10.96	a	10.09	a	2.03	
2-Methylfuran	615	0.78	b	0.83	b[2]	10.70	a	1.36	b[2]	1.50	0.78	b	2.28	a[1]	3.08	a	2.42	a[1]	0.26	
Butanal (44)[C]	622	0.05	b	0.04	b	0.30	a[1]	0.05	b	0.01	0.05		0.03		0.03	[2]	0.03		0.01	
Tetrahydrofuran (42)[C]	643	0.13		0.06		0.39		0.43		0.14	0.13	a	0.05	bc	0.06	b	0.03	c	0.01	
2-Ethylfuran (81)[C]	720	0.07	b	0.15	b[1]	2.95	a[1]	0.32	b	0.47	0.07	b	0.04	b[2]	0.05	b[2]	0.17	a	0.03	
2,5-Dimethylfuran (96)[CD]	726	0.21		0.22		0.24	[1]	0.18		0.03	0.21	a	0.09	bc	0.03	c[2]	0.11	b	0.02	
Pentanal	737	1.76	b	2.36	b[1]	66.68	a[1]	4.66	b	3.80	1.76	b	0.96	b[2]	2.34	b[2]	8.86	a	1.18	
Propanoic acid	807	6.28	a	4.18	b	2.89	b[2]	6.25	a	0.54	6.28	a	3.65	b	3.74	b[1]	4.50	ab	0.56	
1-Pentanol	826	8.78	b	7.50	b	56.49	a[1]	14.14	b	8.58	8.78	b	5.00	c	8.78	b[2]	12.01	a	0.71	
Hexanal	840	15.18	b	14.26	b	991.73	a[1]	35.49	b	26.61	15.18		6.48		20.04	[2]	36.39		7.88	
2-Butylfuran (81)[C]	909	0.07	b	0.07	b	0.66	a[1]	0.08	b	0.01	0.07	b	0.04	c	0.06	bc[2]	0.09	a	0.01	
1-Hexanol	922	15.93	b	19.44	b	128.98	a[1]	23.56	b	8.74	15.93	bc	7.45	c	16.69	b[2]	29.18	a	2.54	
Heptanal (44)[C]	940	0.88	b	0.29	b	3.37	a[1]	1.00	b	0.23	0.88		0.81		1.12	[2]	1.23		0.18	
2-Pentylfuran	1009	1.56	c	2.29	c[2]	17.22	a[1]	13.71	b	0.69	1.56	b	5.59	a[1]	8.02	a[2]	9.24	a	1.25	
1-Heptanol (70)[C]	1023	0.14	b	0.11	b	0.60	a[1]	0.12	b	0.05	0.14		0.10		0.12	[2]	0.12		0.03	
Octanal (43)[C]	1047	0.51	b	0.41	b	1.52	a[1]	0.49	b	0.05	0.51		0.54		0.55	[2]	0.60		0.10	
Hexanoic acid	1074	10.91	b	16.31	b	29.25	a	28.90	a	1.89	10.91	c	20.42	b	24.32	ab	26.60	a	1.98	
2-Ethyl-1-hexanol	1082	3.66	c	4.83	c[2]	8.92	b	18.84	a	1.20	3.66	d	10.15	c[1]	12.77	b	15.67	a	0.73	
1-Octanol (56)[C]	1123	0.14	b	0.13	b	0.31	a[1]	0.11	b	0.01	0.14		0.12		0.12	[2]	0.13		0.01	
Nonanal	1149	9.93	c	8.33	c	28.66	a	17.89	b	1.68	9.93	b	13.36	b	23.88	a	22.29	a	1.69	
Heptanoic acid (60)[C]	1167	0.08	b	0.06	b[1]	0.14	a	0.05	b[1]	0.01	0.08	a	0.04	b[2]	0.03	b	0.03	b[2]	0.01	
Decanal	1256	1.15	b	2.65	b[1]	1.94	b[2]	11.66	a	0.52	1.15	b	1.38	b[2]	7.19	a[1]	9.86	a	0.91	
Octanoic acid	1264	7.80	c	13.69	b	18.81	b[2]	26.61	a	1.57	7.80	b	11.37	b	27.46	a[1]	31.23	a	1.42	
Nonanoic acid	1357	1.41	c	3.16	bc[1]	5.62	b	8.68	a	0.89	1.41	b	1.38	b[2]	8.70	a	10.25	a	1.07	
Decanoic acid	1450	2.47	c	5.88	b[1]	7.63	b[2]	12.89	a	0.61	2.47	c	4.40	b[2]	12.73	a[1]	14.09	a	0.53	
1-Dodecanol[CD]	1523	3.57	b	5.41	b	20.38	a	13.40	a[2]	2.60	3.57	b	4.44	b	24.95	a	30.91	a[1]	2.45	
Bacterial metabolism																				
Lipid β oxidation		**38.67**	**b**	**44.83**	**b**	**86.23**	**a[1]**	**61.67**	**ab**	7.83	**38.67**	**ab**	**30.28**	**b**	**47.53**	**ab[2]**	**51.24**	**a**	5.16	

2-Pentanone	733	7.24		8.13		7.06		7.94		2.10	7.24		5.50		5.12		5.62		1.21
3-Pentanone(57)[C]	740	1.52	b	1.92	b[1]	9.93	a[1]	2.30	b	0.45	1.52	ab	0.75	c[2]	1.30	bc[2]	1.94	a	0.20
4-Heptanone[D]	911	8.07		4.75		10.50	[1]	8.79	[1]	1.22	8.07	a	2.76	c	3.76	bc[2]	5.27	b[2]	0.76
2-Heptanone	933	14.70		21.44		27.54	[1]	18.01	[1]	4.49	14.70		11.90		13.48	[2]	10.64	[2]	2.13
1-Octen-3-ol (57)[C]	1030	0.82	b	1.00	b	13.18	a[1]	2.40	b	2.36	0.82	b	2.02	a	1.91	a[2]	2.29	a	0.23
2-Nonanone	1140	5.67	b	6.01	b	16.35	a	17.42	a	1.25	5.67	b	6.48	b	17.78	a	19.32	a	0.87
2-Undecanone	1346	0.64	c	1.58	b[1]	1.68	b[2]	4.82	a	0.24	0.64	b	0.87	b[2]	4.17	a[1]	6.16	a	0.66
Carbohydrate fermentation		**626.27**	**b**	**776.57**	**a[1]**	**334.02**	**c**	**553.45**	**b**	54.56	**626.27**		**450.50**	**[2]**	**524.12**		**473.48**		55.14
Acetaldehyde	466	8.28	ab	10.73	a	5.72	b[2]	11.30	a	1.23	8.28	b	14.44	a	17.85	a[1]	12.34	ab	1.84
Ethanol	507	95.39		146.86		112.71	[2]	244.50		38.44	95.39	b	212.70	a	219.62	a[1]	221.72	a	24.07
Acetone (43)[C]	529	47.39		49.21		31.93	[2]	45.73		5.65	47.39		52.14		50.60	[1]	49.98		4.58
2,3-Butanedione	626	9.11	ab	12.06	a[1]	2.85	c	6.51	bc	1.75	9.11	a	3.02	b[2]	3.74	b	2.07	b	1.19
2-Butanone	630	27.32	a	22.95	ab[2]	19.03	b[2]	26.07	a[2]	1.74	27.32		32.13	[1]	37.60	[1]	36.48	[1]	3.62
Acetic acid	717	234.68	b	326.72	a[1]	137.81	c	171.96	c	16.91	234.68	a	113.40	b[2]	123.74	b	133.54	b	16.23
3-Hydroxy-2-butanone (45)[C]	782	143.01	a	115.46	a	12.75	b[2]	39.20	b	23.41	143.01	a	16.36	b	65.07	ab[1]	12.51	b	21.35
2,3-Butanediol (45)[C]	891	51.82	b	80.24	a[1]	4.46	c	2.77	c[1]	5.94	51.82	a	2.34	b[2]	1.85	b	0.68	b[2]	4.24
Butanoic acid (60)[C]	889	9.28	b	12.34	a[1]	6.77	c[1]	5.41	c	0.43	9.28	a	3.97	b[2]	4.05	b[2]	4.16	b	0.37
Amino acid degradation		**294.48**	**b**	**366.86**	**a[2]**	**225.60**	**b[2]**	**466.59**	**a[2]**	27.05	**294.48**	**b**	**688.45**	**a[1]**	**675.12**	**a[1]**	**725.91**	**a[1]**	53.35
Methanethiol	472	1.42		1.28		1.75		1.28	[2]	0.22	1.42		1.43		1.64		1.84	[1]	0.13
Dimethyl sulfide (62)[C]	532	0.06	ab	0.04	b[2]	0.09	a	0.06	b[2]	0.01	0.06	b	0.31	a[1]	0.11	b	0.10	b[1]	0.03
2-Methylpropanal	593	3.25		4.99	[2]	2.75	[2]	2.22	[2]	0.79	3.25	b	11.31	a[1]	11.07	a[1]	9.65	a[1]	1.07
2-Methyl-3-buten-2-ol	653	1.23	a	0.83	b	0.60	b	0.83	b[1]	0.13	1.23	a	0.57	b	0.51	b	0.45	b[2]	0.11
2-Methyl-1-propanol	680	24.88	ab	25.83	a	10.45	c[2]	18.21	b	2.19	24.88	a	21.52	ab	15.36	bc[1]	14.52	c	2.47
3-Methylbutanal	689	21.92	ab	28.71	a[2]	16.76	b[2]	29.03	a[2]	3.02	21.92	b	73.60	a[1]	81.54	a[1]	74.02	a[1]	6.14
2-Methylbutanal	700	14.51		18.64	[2]	26.39	[2]	19.15	[2]	3.58	14.51	b	48.02	a[1]	44.05	a[1]	42.12	a[1]	4.63
Dimethyl disulfide	772	2.72	ab	1.81	b	2.16	b	3.74	a	0.32	2.72		1.45		1.97		2.51		0.31
3-Methyl-3-buten-1-ol (56)[C]	789	1.34	b	1.34	b	0.84	c[2]	1.50	a	0.05	1.34		1.26		1.29	[1]	1.37		0.04
3-Methyl-1-butanol	794	147.41	b	200.56	a	104.79	c[2]	174.13	ab	13.06	147.41		196.37		160.06	[1]	160.90		18.96
2-Methyl-1-butanol	796	35.59	bc	51.71	a	27.51	c[2]	41.87	ab	3.85	35.59		51.86		40.05	[1]	42.44		4.33
3-Methyl-2-buten-1-ol	833	12.24		11.88		13.67		15.55		1.07	12.24		13.49		13.52		14.50		1.03
3-Methyl-2-butenal (84)[C]	842	0.25		0.24		0.28		0.33		0.04	0.25		0.26		0.30		0.28		0.03
Methylpyrazine (94)[C]	859	0.52	a	0.43	ab	0.31	b	0.43	ab	0.04	0.52		0.30		0.43		0.35		0.06
2-Methylpropanoic acid (43)[C]	861	6.44	b	2.65	b[2]	1.19	b[2]	26.66	a[2]	5.17	6.44	b	61.10	a[1]	52.46	a[1]	65.04	a[1]	10.33
Ethyl benzene (91)[C]	883	0.34		0.36	[1]	0.44	[1]	0.33		0.03	0.34		0.26	[2]	0.29	[2]	0.34		0.02
3-Methylbutanoic acid (60)[C]	939	11.97	b	5.24	b[2]	2.77	b[2]	85.39	a[2]	5.27	11.97	b	130.36	a[1]	156.60	a[1]	176.80	a[1]	21.31
2,6-Dimethylpyrazine (108)[C]	943	0.86	b	1.48	a[1]	0.37	c[1]	0.41	c	0.09	0.86	a	0.26	b[2]	0.23	b[2]	0.25	b	0.05

2-Methylbutanoic acid (74)[C]	945	1.60	b	1.83	b[2]	1.03	b[2]	23.31	a[2]	3.51	1.60	b	58.44	a[1]	63.06	a[1]	84.40	a[1]	10.73
2-Acetyl-1-pyrroline (43)[C]	960	0.32	c	0.08	d	0.48	a	0.39	b[2]	0.02	0.32		0.65		0.44		0.59	[1]	0.10
3-Methylthiopropanal (48)[C]	966	0.06	b	0.08	b[2]	0.19	a	0.07	b[2]	0.02	0.06	b	0.12	b[1]	0.27	a	0.30	a[1]	0.03
Benzaldehyde	1016	1.76	c	2.13	c	5.31	b[2]	9.33	a	0.43	1.76	b	2.54	b	8.46	a[1]	7.66	a	0.90
3-Thiophenethiol (116)[C]	1047	0.06	b	0.04	b[2]	0.05	b[2]	0.17	a[2]	0.02	0.06	c	0.21	b[1]	0.24	ab[1]	0.27	a[1]	0.01
3-Methylthiopropanol (106)[C]	1061	0.02	b	0.08	a	0.08	ab	0.09	a	0.02	0.02	b	0.20	a	0.13	ab	0.17	a	0.03
Benzene acetaldehyde (91)[C]	1107	0.16	b	0.29	b[2]	0.87	a	0.21	b[2]	0.08	0.16	c	0.59	b[1]	0.83	a	0.87	a[1]	0.06
Phenol (94)[C]	1111	0.96	a	1.29	a[1]	0.88	a[1]	0.34	b[1]	0.14	0.96	a	0.20	b[2]	0.16	b[2]	0.18	b[2]	0.11
Benzyl alcohol (79)[C]	1119	0.06	a	0.06	a[1]	0.02	b	0.03	b	0.01	0.06	a	0.02	b[2]	0.03	b	0.03	b	0.00
Phenylethylalcohol (91)[C]	1192	1.40	b	1.28	b[2]	1.79	b	4.83	a[2]	0.55	1.40	b	10.29	a[1]	10.57	a	13.33	a[1]	2.08
Benzothiazole	1294	1.12	b	1.68	b	1.78	b[2]	6.69	a	0.55	1.12	b	1.46	b	9.45	a[1]	10.63	a	1.39
Esterase activity		**41.96**	**b**	**67.75**	**b[2]**	**61.81**	**b[2]**	**121.65**	**a[2]**	17.93	**41.96**	**b**	**276.60**	**a[1]**	**328.66**	**a[1]**	**342.24**	**a[1]**	36.46
Methyl acetate (43)[C]	552	0.17		0.17		0.30		0.18		0.04	0.17		0.13		0.13		0.19		0.02
Ethyl acetate	635	6.87	c	28.94	a[1]	7.59	c[2]	19.19	b	2.64	6.87	b	11.67	ab[2]	17.86	a[1]	16.07	a	2.37
Ethyl propanoate (102)[C]	744														0.02		0.02		0.00
Ethyl 2-methylpropanoate (43)[C]	788	1.86	b	0.54	b[2]	0.28	b[2]	6.44	a	0.95	1.86		7.59	[1]	9.60	[1]	10.26		2.03
Ethyl butanoate (71)[C]	830	0.55		0.98	[1]	1.00		0.63		0.15	0.55		0.50	[2]	0.52		0.51		0.08
Isobutyl acetate	805	2.68	b	2.09	b[2]	2.42	b[2]	4.09	a	0.40	2.68	b	4.15	ab[1]	3.80	ab[1]	4.95	a	0.56
Butyl acetate (43)[C]	846	0.12	bc	0.27	a	0.23	ab	0.04	c	0.04	0.12		0.15		0.16		0.11		0.05
Ethyl 2-hydroxypropanoate	866	12.19	bc	14.78	b[2]	8.20	c[2]	22.64	a[2]	1.47	12.19	b	196.01	a[1]	203.86	a[1]	213.72	a[1]	25.22
Ethyl 2-methylbutanoate	877	6.11	b	2.43	b[2]	2.12	b[2]	26.42	a[2]	3.15	6.11	c	29.00	b[1]	50.05	a[1]	48.78	a[1]	5.88
Ethyl 3-methylbutanoate (88)[C]	881	0.53	b	0.38	b[2]	0.26	b[2]	7.74	a[2]	0.93	0.53	b	10.61	a[1]	14.62	a[1]	14.19	a[1]	2.93
3-Methyl 1-butanol acetate	906	5.36	a	6.93	a	2.64	b	2.17	b	0.65	5.36	a	4.09	a	1.73	b	5.91	a	0.63
2-Methyl-1-butanol acetate (43)[C]	909	0.21		0.27	[1]	0.19		0.13	[2]	0.03	0.21	a	0.10	b[2]	0.10	b	0.15	b[1]	0.02
Ethyl hexanoate	1028	3.06	b	3.96	b[2]	30.70	a[1]	12.27	b	3.56	3.06	b	9.18	a[1]	10.23	a[2]	9.81	a	0.59
Ethyl octanoate	1229	1.09	c	3.63	b[1]	4.79	b[2]	12.01	a	0.82	1.09	b	1.98	b[2]	9.89	a[1]	11.02	a	0.37
Bornyl acetate	1343	1.16	b	2.39	b	1.09	b[2]	7.68	a	0.52	1.16	b	1.45	b	6.09	a[1]	6.56	a	0.22
Unknown compounds or contaminants		**41.02**	**ab**	**30.36**	**bc[2]**	**23.24**	**c[2]**	**57.72**	**a**	6.08	**41.02**	**b**	**54.56**	**ab[1]**	**72.85**	**ab[1]**	**79.46**	a	9.98
Carbon disulfide	538	38.04	ab	26.86	bc	19.57	c[2]	46.82	a	6.28	38.04		46.35		63.85	[1]	67.97		10.27
Pyridine (79)[C]	785	0.57	a	0.61	a[1]	0.42	b[2]	0.37	b	0.03	0.57	b	0.45	c[2]	0.64	a[1]	0.35	d	0.02
3-Carene (93)[C]	1021	0.07		0.06	[2]	0.09		0.08		0.01	0.07		0.08	[1]	0.08		0.08		0.00
Butyrolactone (42)[C]	1020	0.88	b	0.66	bc[2]	0.46	c[2]	1.97	a[2]	0.09	0.88	b	2.74	a[1]	3.74	a[1]	3.44	a[1]	0.37
D-Limonene (68)[C]	1045	0.28		0.24	[2]	0.35		0.37		0.04	0.28	b	0.35	b[1]	0.38	ab	0.50	a	0.05
p-Cymene	1050	0.65	c	1.40	b[2]	1.83	b	7.54	a[1]	0.20	0.65	c	3.96	b[1]	3.53	b	6.50	a[2]	0.48
Dimethylsulfone (79)[C]	1060	0.29		0.34		0.26		0.26		0.02	0.29	a	0.28	ab	0.23	bc	0.21	c	0.02

4-Methylphenol (107)[C]	1196	0.24	0.19 [2]	0.28	0.31 [2]	0.04	0.24 b	0.34 a[1]	0.40 a	0.42 a[1]	0.03

AU: Abundance units, the result of counting the total ion chromatogram (TIC) for each compound.

[A] Linear retention index (LRI) of the compounds eluted from the GC-MS using a DB-624 column capillary column (30m x 0.25mm i.d. x 1.4µm film thickness).

[B] Different letters in the same row of each group (uninoculated and inoculated) indicate significant differences at $p<0.05$ among batches. Different numbers superscript indicate significant differences at $p<0.05$ between uninoculated and inoculated samples within the same reformulation.

[C] Target ion used to quantify the compound when the peak was not completely resolved.

[D] Tentatively identification by mass spectrum.

Table 3. Odor active compounds identified in the HS of dry fermented sausages.

Compound	LRI[A] compound	LRI[A] standard	Descriptor
Lipid autooxidation			
Propanoic acid	813	802	Glue, wax
Hexanal	835	836	Fresh cut grass
2-Pentylfuran	1011	1011	Metallic, green, unpleasant, cabbage
Octanal	1046	1047	Lemon, floral
Decanal	1245	1254	Green wood
Nonanoic acid	1357	1347	Spicy, hazelnut, walnut
Bacterial metabolism			
Lipid β-oxidation			
3-Pentanone	739	739	Butter, damp
1-Octen-3-ol	1025	1028	Mushroom
2-Nonanone	1139	1142	Toasted
Carbohdrate fermentation			
2,3-Butanedione	632	632	Butter, cooked ham
Acetic acid	695	700	Vinegar
Amino acid degradation			
Methylpyrazine	852	858	Green, cooked potato
3-Methylbutanoic acid	924	926	Strong cheese
2-Acetyl-1-pyrroline	962	960	Savoury, snacks
3-Methylthiopropanal	968	969	Cooked potato or vegetables
Benzaldehyde	1016	1021	Toasted, tobacco
Phenol	1113	1112	Wet dog hair
Benzothiazole	1293	1305	Toasted, savoury, sulfurous, pepper
Esterase activity			
Ethyl 2-methylpropanoate	786	789	Sweet, strawberry
Ethyl 2-methylbutanoate	871	872	Sweet, strawberry
Ethyl 3-methylbutanoate	875	876	Fruity, floral, sweety
Unknown compounds			
Unknown 1	1032		Fish, amines, metallic, herbal
Unknown 2	1178		Savoury, snacks, toasted almond
Unknown 3	1186		Sulfurous, cooked vegetable, burnt
4-Methylphenol	1193	1190	Burnt plastic, dung
Unknown 4	1203		Unpleasant, plastic, rubber
Unknown 5	1218		Green, freshness

[A] Linear retention index (LRI) of the compounds or standards eluted from the GC-FID-O using a DB-624 capillary column (60m x 0.32mm x 1.8µm).

Figure 1

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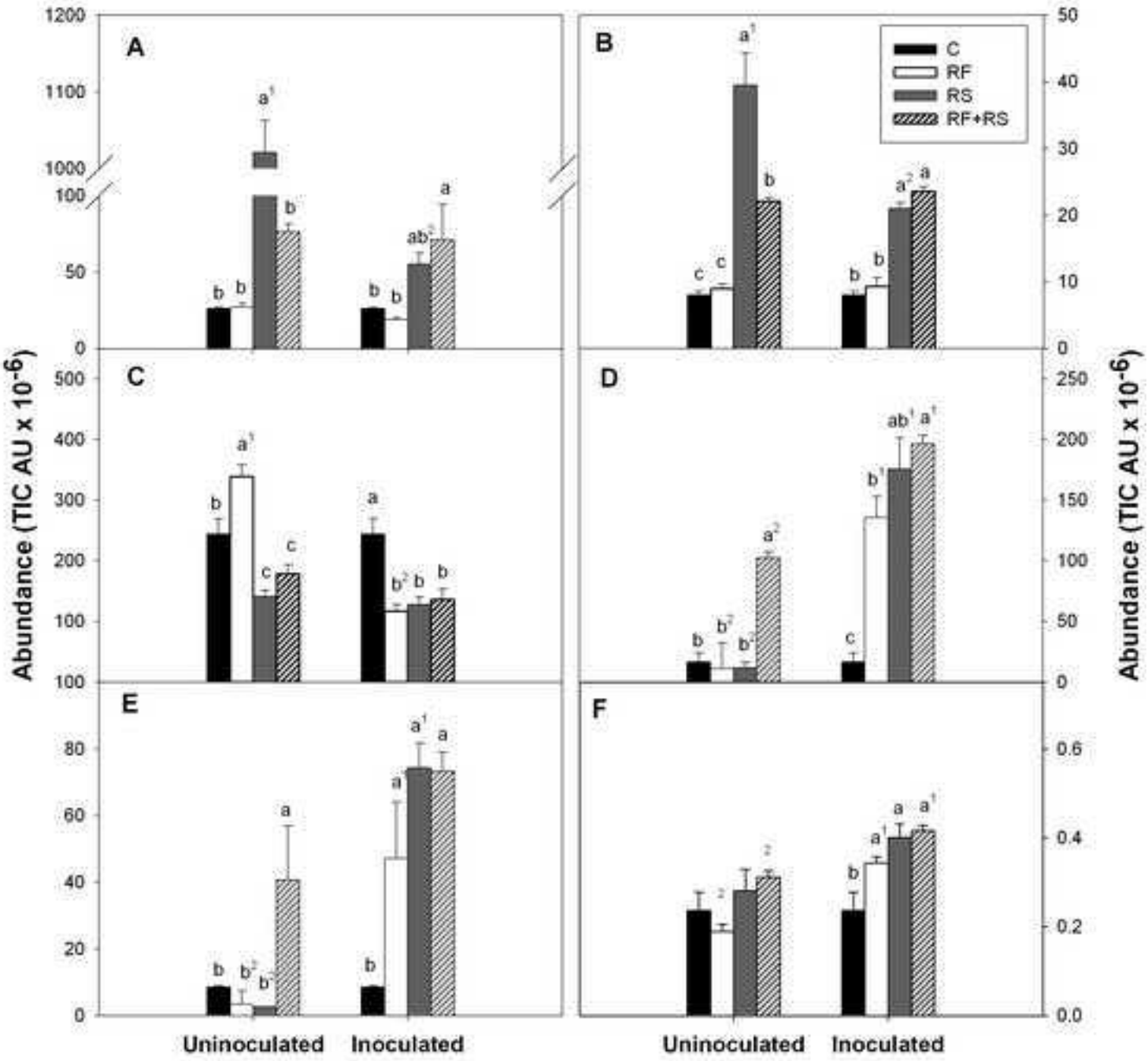
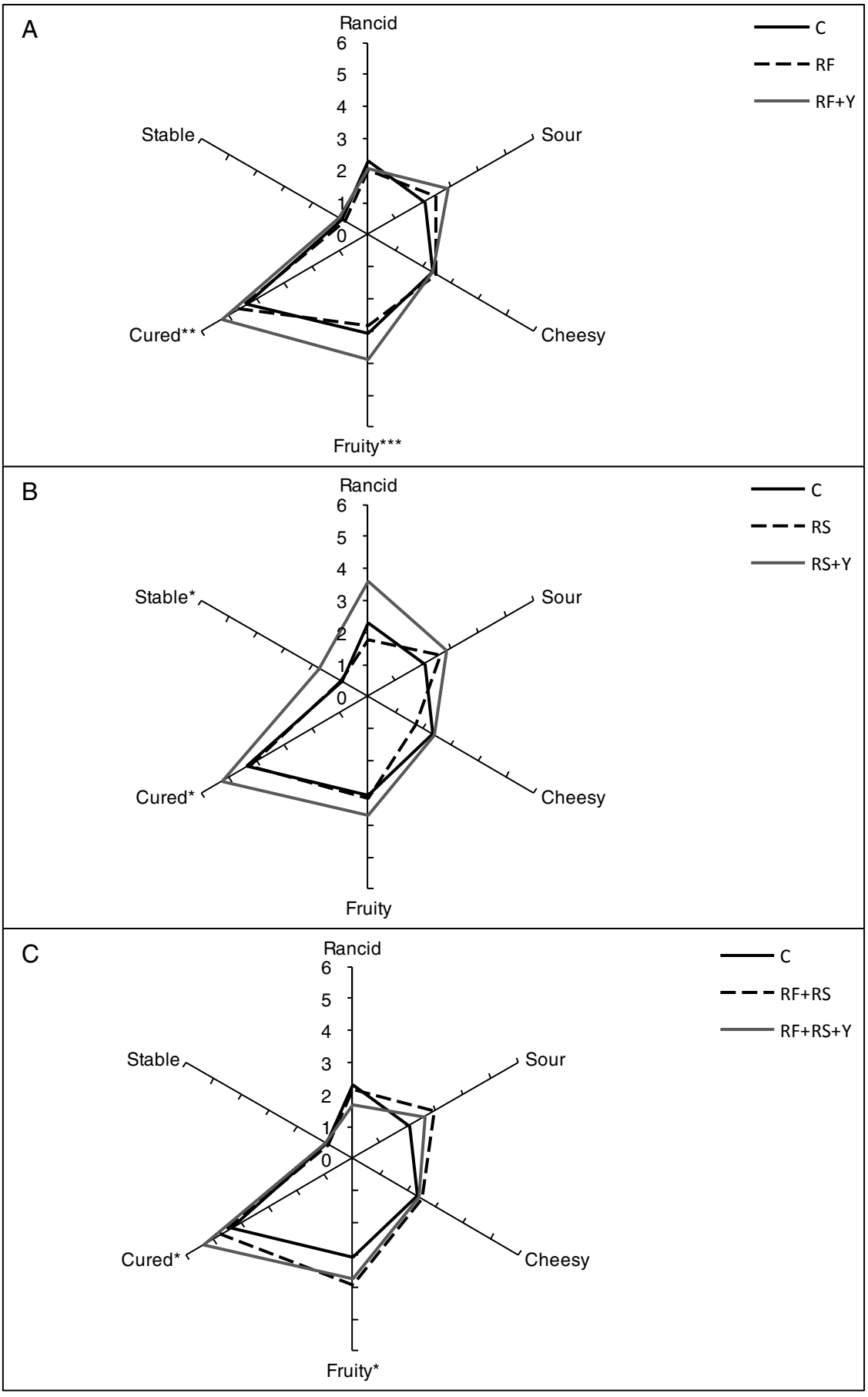


Figure 2


A
Rancid
6
5
4
3
2
1
0
Stable
Sour
Cured**
Cheesy
Fruity***
C
RF
RF+Y
B
Rancid
6
5
4
3
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1
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Stable*
Sour
Cured*
Cheesy
Fruity
C
RS
RS+Y
C
Rancid
6
5
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3
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1
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Stable
Sour
Cured*
Cheesy
Fruity*
C
RF+RS
RF+RS+Y

Figure 3

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