

**Lipolysis and aroma generation as mechanisms involved in masking
boar taint in sodium reduced fermented sausages inoculated with *D.*
hansenii yeast**

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Running title: Lipolysis and aroma development in boar fermented sausages.

ABSTRACT

BACKGROUND: The use of boar back fat for processing of fermented sausages may cause the presence of abnormal odours. In dry-cured products, ripening time is essential to develop the sensory characteristics. Yeast has been proposed as an alternative to mask boar taint odour through its metabolic activity but it is necessary to elucidate which mechanisms are involved. The aim is to study the effect of *D. hansenii* inoculation on the lipolysis process and generation of aroma compounds in fermented sausages manufactured with boar back fat at two different ripening times.

RESULTS: *D. hansenii* inoculated sausages had a higher degree of lipolysis as demonstrated by higher content of free fatty acids, ester compounds and branched aldehydes which contribute the fruity odour. The increase in lipolysis produced by *D. hansenii* inoculation was not followed by an increase in oxidation during processing possibly due to the metabolic activity of yeast. The effect of back fat type was scarcely appreciated whereas ripening time had a stronger effect on sausage. Boar sausages were characterized by a lower polyunsaturated fatty acid profile and lesser lipolysis than gilt sausages.

CONCLUSION: Yeast inoculation with *D. hansenii* and long ripening time were appropriate strategies to limit the perception of boar taint in dry fermented sausages.

Keywords: yeast, boar taint, aroma, fermented sausage, lipolysis, volatile.

INTRODUCTION

Surgical castration of pigs is an animal welfare concern but it is practiced to avoid undesirable sexual odour or aggressive behaviour¹. The European declaration on alternatives to surgical castration of pigs and the voluntary agreement to stop surgical castration by 1st of January 2018 have produced a shift in the production chain to entire male. As indicated in the First progress report from the above European declaration,² an increase in the percentage of non-castrated male pigs has been reported especially in Belgium, The Netherlands, Germany, Spain, and France since 2006 until 2014. In this sense, Borrissier-Pairó et al³ concluded that the percentage of carcasses with high levels of androstenone and/or skatole in commercial pigs from Spanish farms is only of 10.2%, but in terms of carcasses per year represents around 1.6 million. In order to give carcasses an adequate use, carcasses should be classified according to the presence of boar taint compounds.³ It is generally accepted that boar taint perception would be less perceived in processed pork products than in fresh meat because it is assumed that processing conditions may mask the boar perception. A similar reduction in the negative odour perception would occur in meat products that are consumed cold where odour release is minimized.⁴ Even though the high fat content of several pork products may increase the risk of boar taint perception.

Current studies are trying to determine the impact that the use of boars has on consumer's perception, especially when different quantities of boar meat are used in different meat products⁵. These studies revealed that consumers detected the presence of sweaty and strong flavours in boiled sausages but not in fermented sausages. The reason seemed to be that the technological process used in North European fermented sausages

including a smoking process, addition of spices and the fermented aroma produced during processing, suppresses the perception of boar taint.

On the contrary, in Mediterranean style fermented sausages, the use of boar back fat was demonstrated to present abnormal odours and other negative sensory characteristics such as elevated hardness and low oxidation values.⁶ In this sense, the relationship between boar taint compounds and fatty acid composition was recently studied⁷ and reported that the content of deposited androstenone and skatole in pigs may be affected by lipid metabolism. Namely, the mentioned study showed increased levels of PUFA in subcutaneous fat tissue of boars with low androstenone, skatole and indole levels. However, the authors could not explain the effect of the variable lipid content and fatty acid composition on flavour formation and boar taint release from subcutaneous tissue. Furthermore, in dry fermented sausages not only lipid content would affect flavour release but also microbial processes essential to obtain the final sensory characteristics.⁸

Previous studies indicated that yeast may be an alternative to mask boar taint odour as they are involved in different biochemical mechanisms releasing aroma compounds.⁹ The effectiveness of *D. hansenii* yeast to mask boar taint by providing fruity flavours and less oxidized sausages together with a reduction in hardness was demonstrated by sensory analysis.¹⁰ Therefore, the aim of the present study was to determine the effect of *D. hansenii* yeast on the lipolysis process and the generation of aroma compounds in dry fermented sausages with reduced sodium content and manufactured with boar back fat at two different ripening times.

MATERIAL AND METHODS

Dry fermented sausages.

Formulations (6 kg/formulation and a total of 24 kg/replicate) of dry fermented sausages were manufactured with back fat from gilt or boar with reduced sodium content (25%) using potassium chloride as a substitute.¹⁰. The sausage formula was: 50% ground pork meat, 50% ground pork back fat from gilt or boar, 20g Kg⁻¹ lactose, 20 g Kg⁻¹ dextrin, 7 g Kg⁻¹ glucose, 20.3g Kg⁻¹ sodium chloride (NaCl), 6.7 g Kg⁻¹ potassium chloride (KCl), 0.5 g Kg⁻¹ sodium ascorbate, 0.15 g Kg⁻¹ sodium nitrite and 0.15 g Kg⁻¹ potassium nitrate. Four different formulations of dry fermented sausages were manufactured: control formulation with back fat from gilt (GS), formulation with boar back fat (MS) and the same two formulations, gilt back fat (GS+Y) and boar back fat (MS+Y) inoculated with *Debaryomyces hansenii* yeast. Sausages were dried for 63 days at 10°C and 70-85 % relative humidity (RH).

The mixture for dry fermentation sausages was prepared using 50% ground pork meat and 50% ground pork back fat from gilt or boar. The pork's ham lean and fat (boneless and skinless) from twelve different animals per sex were purchased from a local producer (Incarlopsa, Spain) and previously chopped and mixed due to variations in androstenone and skatole contents to achieve a homogeneous mass. Yeast was cultivated as described in Corral et al¹⁰ and appropriate volumes of yeast strain *D. hansenii* P2 suspension¹¹ were added to the inoculated batches at final concentration of 5 x 10⁶ c.f.u. g⁻¹ of meat batter.

Four different formulations (fat types and yeast) were manufactured and each one was replicated three times obtaining a total of 12 batches (3 x 2 x 2). From each batch, two sausage samples were randomly chosen at different ripening times 0, and after 43 and 63 d of processing¹⁰. Casing was removed from sausage and a sausage sample was vacuum packed in aluminium foil and frozen at -80°C for lipid, volatile and aroma analysis.

Lipid profile and lipolysis.

Total lipids were extracted from 5 g of sausage according to Folch et al¹² using dichloromethane:methanol (2:1) instead of chloroform:methanol (2:1) as solvent. The extracts were dried in a rotating vacuum evaporator and weighed to determine the total quantity of lipids. Lipid profile was determined by means of total fatty acids which were methylated as described by Berry et al¹³. Fatty acids methyl esters (FAME) were analysed in an Agilent HP 7890B gas chromatograph (GC) equipped with a flame ionisation detector (FID).¹⁴ For quantification, response factors of the standards respect to an internal standard (C21:0) were calculated using the standard fatty acid methyl ester solution (FAME mix, Sigma-Aldrich, Germany). The results were expressed as percentage of total fatty acids identified.

Lipolysis was tested by analysis of free fatty acids (FFA) released throughout ripening process. The free fatty acids were separated from the lipid fraction using an ion exchange resin.¹⁵ FFAs were converted into fatty acid methyl esters (FAME) using boron fluoride-methanol (Sigma-Aldrich, Chemical Co., Milwaukee, WI) as the methylation reagent¹⁶ and analysed in a GC-FID.¹⁴ Quantification was performed using response factors as indicated above. The results were expressed as mg of fatty acid 100 mg⁻¹ of dry fermented sausage in dry matter.

Aroma analysis

Profile and quantification of volatile compounds

The analysis of volatile compounds in the headspace (HS) of dry fermented sausage was performed by solid phase micro extraction and gas chromatography and mass spectrometry analysis (SPME-GC-MS).¹⁴ Five grams of the minced sausage was

weighted into a 20 ml HS vial sealed with a PTFE faced silicone septum and 0.75 mg of butylhydroxytoluene (BHT) was added. The vial was equilibrated at 37°C for 30min and then, SPME fibre (CAR/PDMS) was exposed to the HS during 2h at 37°C. The volatile compounds were desorbed in port injection of GC-MS (HP 7890A/5975C) (Hewlett Packard, Palo Alto, CA) for 5min at 240°C (in splitless mode) and equipped with a Gerstel MPS2 multipurpose sampler (Gerstel, Germany). The volatile compounds were separated using a DB-624 capillary column (J&W Scientific, Agilent Technologies, USA) and identified by comparison with mass spectra from the library database (Nist'05), calculating Kovats retention index¹⁷ by using the alkane standard solution C8-C20 (Sigma-Aldrich, Madrid, Spain) and by comparison with authentic standards. The identified volatile compounds were quantified in SCAN mode using either total or extracted ion chromatogram (TIC or EIC) on an arbitrary scale. The results were expressed as abundance units (AU) 10⁻⁶.

Olfactometry analysis (GC-O)

The analysis of aroma compounds extracted by SPME was performed using a gas chromatograph (Agilent 6890, USA) equipped with a FID and sniffing port detectors (ODP3, Gerstel, Mülheim an der Ruhr, Germany).¹⁶ Each assessment was carried out according to Olivares et al.¹⁶ Four trained panellists evaluated the odours from the GC-effluent of the ripened sausages (63 days). The detection of an odour by less than three assessors was considered to be noise. The panellists were selected among the staff of the institute based on their ability to detect and recognize odours and experienced in previous olfactometry studies.

Compounds were identified using the following techniques: comparison with mass spectra, comparison with kovats retention indices of authentic standards injected in the

GC-MS and GC-O, and by coincidence of the assessor's descriptors with those in the Fenaroli's handbook of flavour ingredients.¹⁸

Statistical analysis

Data were analysed using Generalized Linear Model (GML) procedure of statistical software (XLSTAT 2011, v5.01, Addinsoft, Barcelona, Spain). The model included the effect of fat type and yeast inoculation as fixed effects and replicates as random effects. When significant effect of the treatment group was detected ($P < 0.05$), least squares means (LSM) were compared using Tukey's test. In addition, principal component analysis (PCA) was performed to evaluate the relationships among aroma compounds, free fatty acid generated and formulations. The aroma compounds abundance and free fatty acids were used as parameters and the sensory odour intensities were used as supplementary variables.

RESULTS AND DISCUSSION

Approximate chemical composition of sausages and levels of boar taint compounds androstenone and skatole have been reported previously in Corral et al.¹⁰

Lipid profile and lipolysis.

The total fatty acid profile was analysed in order to determine the effect of back fat type on the manufactured sausages (Table 1). As reported previously, the use of boar back fat was associated with lower batter fat content that resulted in harder and less oxidised (TBARS values) sausages.¹⁰ However, differences in terms of lipid profile in the batters to establish their effect on oxidation and generation of aroma compounds were not explained. Table 1 shows significantly lower content of polyunsaturated fatty

acids (PUFA) in boar than gilt sausage formulations that can explain the lower susceptibility to oxidation of the boar formulations in addition to their lower fat content. This is in agreement with a previously mentioned study⁶ where the effect of sodium reduction together with the use boar back fat in fermented sausages was determined. In contrast, our results showed no differences between boar and gilt sausage formulations in saturated (SFA) and monounsaturated fatty acids (MUFA) content. A higher PUFA content is generally reported in boar than gilts.¹⁹ Different results in present study may be due to the dietary treatments (that was not controlled) as it has a higher impact than sex on PUFA content.²⁰

Recently, Mörlein & Tholen⁷ suggested that the levels of deposited androstenone and skatole may be affected by lipid metabolism in pigs. They found different fatty acid composition in subcutaneous tissue of boars depending on the levels of boar taint compounds, and observed that boar pigs with low levels of boar compounds display higher PUFA content. However, they did not clarify how this could affect flavour formation and boar taint release from subcutaneous tissue. In this sense, flavour formation in fermented sausages seems to be a complex process where not only matrix composition has an important role but also key microbiological processes are involved such as the fermentation of carbohydrates by lactic acid bacteria and the microbial activity of Coagulase-negative staphylococci (CNS) and yeasts.²¹ Nevertheless, levels of boar taint compounds in sausages' melted fat at 43 and 63 d were analysed as reported in Corral et al.¹⁰ Boar sausages presented high levels of androstenone (383–885 ng g⁻¹ melted fat) while this steroid was not present in gilt back fat sausages. Regarding indole and skatole compounds, both were present in melted fat from boar (22-81 ng g⁻¹ indole and 143-285 ng g⁻¹ skatole) as well as from gilt sausages (47-95 ng g⁻¹ indole and 250-344 ng g⁻¹ skatole). In the mentioned study,¹⁰ androstenone levels in boar sausages

were above its threshold ($0.5\text{--}1\ \mu\text{g g}^{-1}$) while skatole levels were close to its threshold of $200\text{--}250\ \text{ng g}^{-1}$. The presence of both compounds in boar sausages indicates an effect on odour perception in contrast to the sole presence of skatole in gilt sausages.

As shown by free PUFA content, using boars affected the lipolysis process (Table 2). At 63 d gilt sausages (GS) had a significant higher content of free PUFA than boar sausages (MS). However, this result was significant only between *D. hansenii* inoculated batches at 43 d (Table 2). The effect of the higher lipolysis observed in gilt sausage was demonstrated in the higher abundance of C18:2n6 and C20:2n6. This would confirm that although the muscle lipolytic enzymes are activated in the gilt sausages, microbial lipolytic enzymes may also contribute.²² Accordingly, the addition of yeasts could also affect the lipolysis. Results in Table 2 seem to confirm that the generation of free fatty acids during sausage fermentation is affected by yeast inoculation. At 43d, yeast inoculation increased significantly the lipolysis in gilt sausages as seen in the free SFA content. In contrast the increase was not significant in MUFA and PUFA although several fatty acids were significantly increased such as C16:1 and C18:3n3. Nevertheless, the effect of *D. hansenii* inoculation on lipolysis was more pronounced at 63 d. In this case, yeast inoculation produced an increase in the content of free MUFA, PUFA and total free fatty acids in the boar sausages, while the increase was not significant in gilt sausages. With regard to the role of yeast in increasing the lipolysis process in fermented sausages, our results agree with previous studies.^{23,14} In fact, the environmental conditions especially the effect of lipid, salt and lactic acid contents affect the expression of lipolytic enzymes in yeasts such as *Y. lipolytica*.²⁴ These environmental factors individually or in combination may affect the lipase expression or activity and produce higher differences than those observed using different strains.²⁴ In our study, the reduced sodium content of sausages might have

affected the lipolysis, however all formulations were reduced in sodium so no differences may be attributed to it. Regarding lipid content, the highest concentration in gilt sausages may produce a stimulant effect on yeast lipase activity as Guerzoni et al indicated.²⁴ However, our results did not show differences in free fatty acids between inoculated gilt and boar formulations as these environmental factors have been shown to produce less than 20% changes in free fatty acids.²⁴

Aroma analysis.

A total of 100 compounds were identified in the sausages and corresponded to 21 aldehydes, 7 alkanes, 8 ketones, 11 acids, 3 nitrogen compounds, 9 sulphur compounds, 12 alcohols, 16 esters, 4 furans, 1 lactone and 8 aromatic hydrocarbons (Table 1 supplementary material). Table 3 shows a summary with the most abundant and those aromatic compounds detected by GCO in the sausage formulations at both ripening times.

Figure 1 represents the abundance of all identified compounds classified by chemical class and the most abundant compounds were aldehydes, acids, alcohols, ketones and ester compounds. In general, production of aldehydes, ketones and acids was significantly reduced while production of alcohols and esters was clearly favoured by *D. hansenii* inoculation independently of the fat type used.

Among aldehydes, the most abundant were hexanal, pentanal, octanal and nonanal (Table 3). At 63d the use of boar back fat seemed to increase the abundance of linear aldehydes (pentanal, hexanal, (E)-2-hexenal, heptanal) although this appeared to be in opposition to the reported TBARS values on the same sausages.¹⁰ These differences were masked by inoculation of *D. hansenii* and inoculated sausages displayed significantly lower aldehyde content in accord with the low TBARS values of these

sausages.¹⁰ The largest reduction in aldehydes was observed in pentanal and hexanal, derived from the lipid oxidation process,²⁵ as well as in 3-methylbutanal, derived from the degradation of the amino acid leucine.¹¹ In contrast 2-methylpropanal derived from valine degradation increased in *D. hansenii* inoculated sausages.

The effect of back fat type and yeast inoculation on alkanes generation was negligible, as few differences were observed after 43 d or 63 d of processing (Table 1 supplementary). Moreover, the aroma impact of these alkanes in sausages seems to be very low, as already found in previous studies.²¹

Regarding ketone compounds, the use of boar back fat resulted in a reduction of 3-hydroxy-2-butanone and 2,3-butanedione as compared to gilt sausages. These differences were counteracted by *D. hansenii* inoculation at both ripening times. In fact, the content of 2-butanone, 3-hydroxy-2-butanone and 2-heptanone decreased and no differences in terms of back fat type were detected (Table 3).

Similarly, the content of acid compounds was different in boar or gilt back fat sausages. The former presented the lowest content of acetic and butanoic acids at 43 d and 63 d. Yeast inoculation decreased the generation of both acid compounds while less abundant acids, generated from the degradation of amino acids such as 2-methylpropanoic and 3-methylbutanoic acids, were produced in higher amounts (Table 3).

Regarding nitrogen compounds, the highest content was detected at 63 d. The type of back fat did not affect their generation, while yeast inoculation produced a significant decrease of 2-acetyl1-pyrroline (Table 3).

Potent sulphur aroma compounds like methional^{26,27,28} were also affected by the use of boar fat. The highest methional content was detected in boar sausages at both ripening times. Yeast inoculation increased the production of sulphur compounds especially due

to the significant raise of carbon disulphide although methional content was reduced (Table 3).

The production of alcohols was significantly higher in *D. hansenii* inoculated sausages independently of the type of back fat used. This was mainly due to the generation of ethanol and methyl branched alcohols, 2- and 3-methyl-1-butanol, derived from the degradation of Ile and Leu, respectively (Table 3).

Yeast inoculation produced an increase in the generation of many ester compounds, as already found in previous studies.^{11, 14} Yeast inoculated gilt back fat sausages had a higher ester concentration than the boar sausages due to an increase in ethyl acetate, ethyl butanoate and ethyl 2-hydroxypropanoate. This is in agreement with the highest generation of ethanol observed in the inoculated gilt sausages.

The effect of fat type on furan compounds generation was more pronounced with longer ripening time, as larger amounts of furans were detected in boar than in gilt sausages at 63 d. In line with aldehydes, ketones and acids production findings, furan compounds decreased due to *D. hansenii* inoculation particularly 2-pentylfuran. This may be due to the inhibitory effect of yeast inoculation on lipid oxidation, in agreement with the lowest TBARS values found in these batches.¹⁰ Furthermore, inhibition of lipid oxidation and decrease of 2-pentyl furan masked the effect of the use of boar fat at both ripening times (Table 3).

Finally, generation of aromatic hydrocarbons was not clearly affected by either fat type or yeast inoculation (Table 3).

In summary, the use of boar back fat resulted in lower production of ketones and acid compounds which was significant at longer ripening time (63d). Taking into consideration that gilt sausages contain more fat¹⁰ and a high PUFA profile they should be more susceptible to oxidation, as observed by the highest abundance of ketones and

acid compounds. However, *D. hansenii* inoculation seemed to inhibit the oxidation process and no differences in terms of oxidation compounds (aldehydes, ketones, acids) were detected regardless of the type of fat used. In contrast *D. hansenii* inoculated gilt sausages were characterized by higher amounts of ester compounds derived from amino acids metabolism and by lower abundance of lipid oxidation products. Present results also indicate that under reduced sodium concentrations yeasts are able to produce aroma compounds.

Since not all identified volatile compounds produce an effect on the aroma, an olfactometry analysis revealed the presence of 30 different aroma notes in the headspace of fermented sausages. Among them, the compounds contributing a higher number of aroma notes were aldehydes and ester compounds responsible for green-fresh and fruity-sweet odours, respectively (Table 4). Additionally, several acids contributed to sour and cheesy notes related to the fermentation process,²¹ alcohols to floral and mushroom odours and several of the most potent aroma compounds, nitrogen and sulphur compounds, to savoury notes.^{29,30} Although all odorants have been previously detected as key odour compounds in fermented sausages^{28,16,26,27,29,30}, no odours related to boar compounds were detected in the headspace of the sausages probably due to the low temperature used for analysis (37°C) as fermented sausages are consumed at room temperature.

Potent odorants such as methional (boiled potato) and 2-acetyl-1-pyrroline (savoury)²¹ were the most affected by yeast inoculation which produced a strong reduction in both compounds. In addition, production of the aldehydes was reduced in inoculated sausages contributing to a decrease in rancid notes (hexanal, (E)-2-hexenal, heptanal) and acetic acid (vinegar) and an increase in fruity and sweet compounds such as ester compounds (ethyl acetate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methyl

and 3-methyl butanoate and ethyl hexanoate) and cheesy compounds (2-methylpropanoic and 3-methylbutanoic acids). The increase in fruity, cheesy odours, and reduction in green and rancid notes appeared to modulate flavour in *D. hansenii* inoculated sausages.

The effect of back fat type and *D. hansenii* inoculation on lipolysis and aroma was evaluated by a principal component analysis using free fatty acids (SFA, MUFA, PUFA and total) and those volatile compounds producing aroma as determined by olfactometry analysis (figure 2). The sensory differences among sausage formulations were analysed by free choice profile.¹⁰ As the panellists quantified the perceived attributes, those attributes related to aroma notes and with the highest number of times reported were selected (cured, rancid, animal and fruity odours). Then, the mean of each odour attribute was used as supplementary variable in the PCA analysis. Two principal components were able to explain 76 % of the variability observed. The most important variable was F1 that explained 55 % and was related to *D. hansenii* inoculation while F2 accounted for 20 % of the variability and was related to ripening time and in lesser extent to the type of back fat used. *D. hansenii* inoculated sausages appeared on the negative side of F1 and were related to a higher lipolysis due to the presence of free fatty acids as well as production of ester compounds and branched aldehydes and finally to the fruity odour. This indicated that *D. hansenii* was responsible of a significant amino acid degradation and further esterification, in addition to a high lipolytic activity. In contrast, non-inoculated sausages appeared related to aroma compounds produced from lipid oxidation reactions; although lipolysis was low in these sausages and related to odours like rancid and animal odours. Moreover, the effect of back fat type was scarcely observed in F2, whereas ripening time seemed to have a stronger effect on sausage separation. Regarding animal odour perception it was related to shorter ripening

times and with those non inoculated sausages what confirms the role of yeast inoculation in decreasing the boar taint perception. Levels of boar taint compounds in sausages' melted fat were studied by Corral et al¹⁰ whom reported androstenone and skatole levels close to their thresholds at both ripening times.

In conclusion, the mechanisms involved in limiting the perception of boar taint in dry fermented sausage were the generation of aroma compounds and the lipolysis process as affected by the use of boar back fat. The increase in lipolysis produced by *D. hansenii* inoculation was not followed by an increase in oxidation during processing possibly due to the metabolic activity of yeast. The lowest content of polyunsaturated fatty acids (PUFA) in boar formulations explained the lowest susceptibility to oxidation of the boar sausages in addition to their lowest fat content. Inoculation of *D. hansenii* was able to mask the differences in aroma development and oxidation produced by the use of boar versus gilt back fat in sodium reduced fermented sausages.

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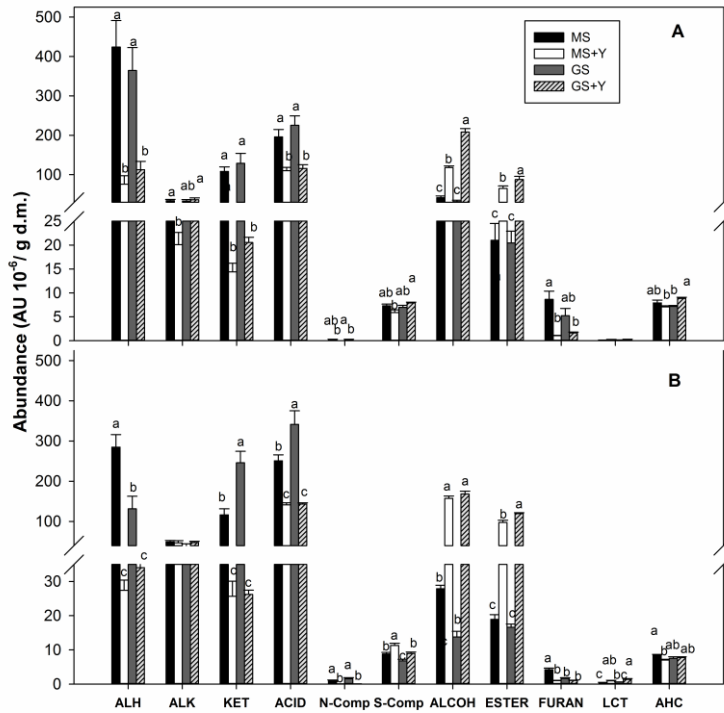
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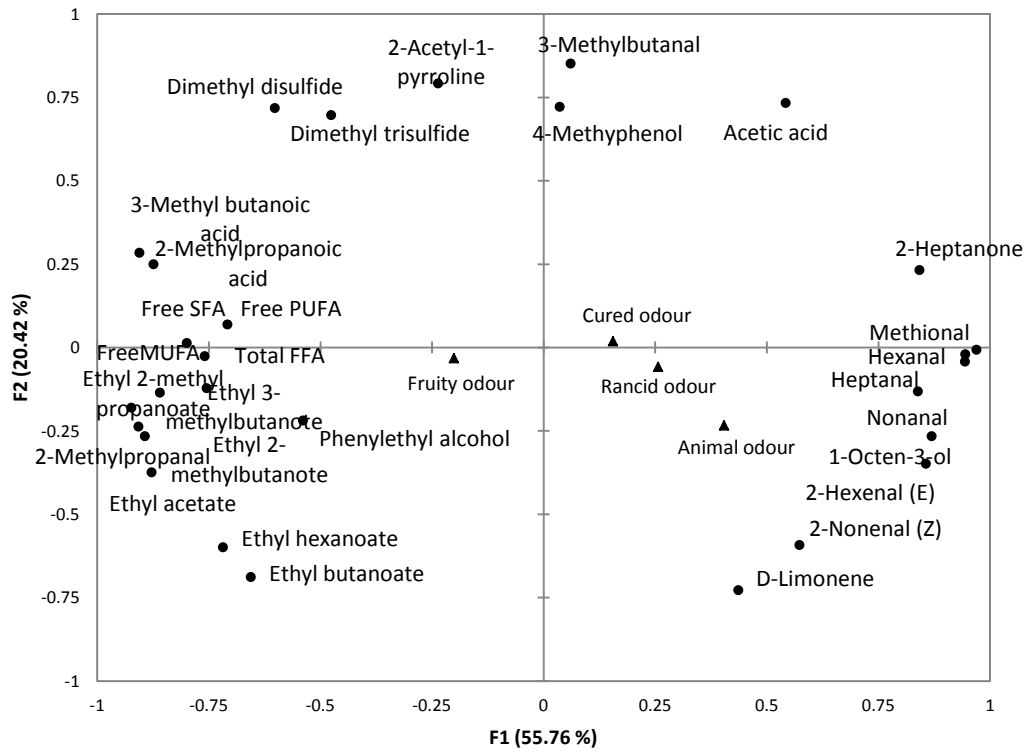
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FIGURE LEGENDS

Figure 1. Effect of back fat (from gilt or boar) and yeast inoculation on volatile compound (classified by chemical class) generated in dry fermented sausages after 43 (A) and 63 d (B) of processing. ALH: aldehydes, ALK: alkanes, KET: ketones, ACID: acids, N-Comp: nitrogen compounds, S-Comp: sulphur compounds, ALCOH: alcohols, ESTER: esters compounds, FURAN: furans, LCT: lactones, and AHC: aromatic hydrocarbons. Different letters in chemical class indicate significant differences among formulations.



F1 and F2: 76.18 %



F1 and F2: 76.18 %

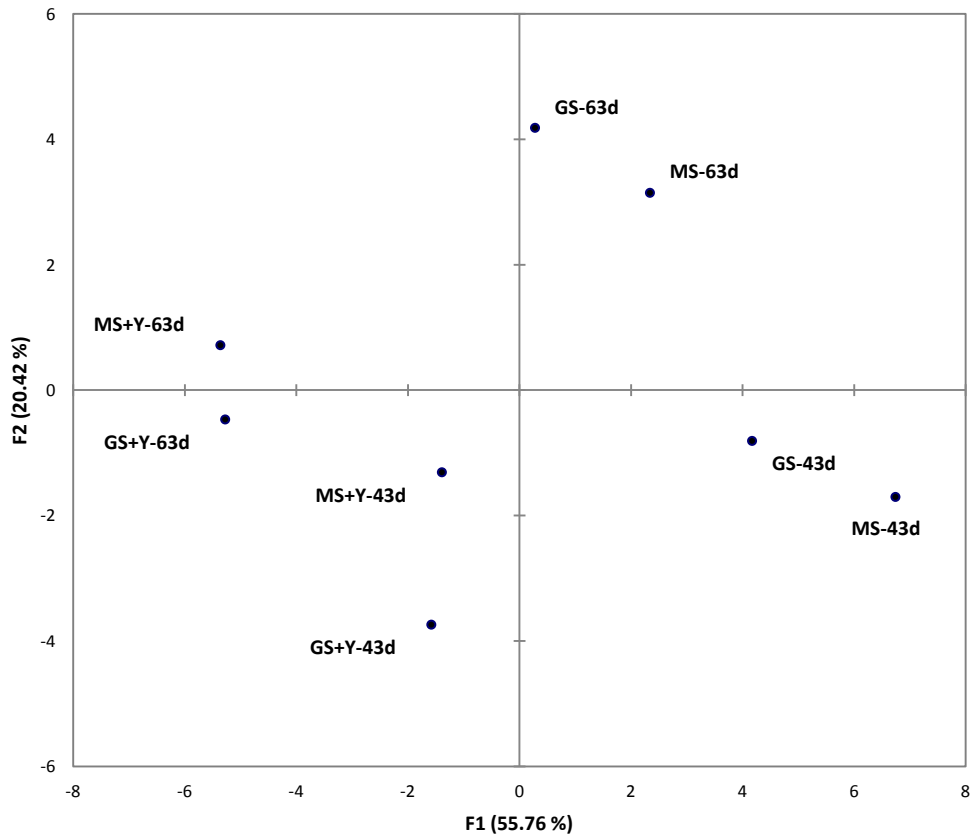


Figure 2. PCA of aroma compounds and free fatty acids generated in dry fermented sausages after 43 and 63d of processing when using entire male back fat and yeast inoculation. Sausages manufactured with boar back fat (MS) and inoculated with *D. hansenii* yeast (MS+Y); Sausages manufactured with gilt back fat (GS) and inoculated with *D. hansenii* yeast (GS+Y). (▲) Odour descriptors and (●) volatile compounds and free fatty acids.

Table 1 . Total fatty acid profile (%) in dry fermented sausages at initial time (0 d) manufactured with boar and gilt back fat with and without yeast inoculation.

Fatty acid	MS	MS+Y	GS	GS+Y	RMSE ¹	P _f ²	P _y
C12:0	0.12 a	0.12 a	0.10 b	0.10 b	0.007	***	ns
C14:0	1.69 a	1.62 a	1.50 b	1.50 b	0.061	***	ns
C16:0	25.52	25.77	25.73	25.79	0.867	ns	ns
C17:0	0.43 a	0.41 a	0.28 b	0.27 b	0.019	***	ns
C18:0	11.75	13.04	12.07	12.45	1.648	ns	ns
C20:0	0.12	0.13	0.13	0.15	0.022	ns	ns
SFA	39.70	41.16	39.85	40.27	2.465	ns	ns
C16:1	2.66	2.53	2.54	2.50	0.128	ns	ns
C17:1	0.33 a	0.30 b	0.20 c	0.18 c	0.014	***	**
C18:1	41.98	40.02	41.00	40.04	1.745	ns	ns
C20:1 n9	0.79	0.97	0.76	0.74	0.228	ns	ns
MUFA	46.04	44.08	44.61	43.60	1.799	ns	ns
C18:2 n6c	12.13 c	12.57 bc	13.51 ab	13.91 a	0.657	***	ns
C18:3 n3	0.64	0.64	0.64	0.68	0.037	ns	ns
C20:2 n6	0.49 b	0.52 b	0.57 a	0.59 a	0.030	***	ns
C20:3 n6	0.04 a	0.04 ab	0.03 b	0.04 ab	0.008	*	ns
C20:4 n6	0.56	0.59	0.52	0.62	0.067	ns	ns
C22:4 n6	0.17 ab	0.17 a	0.12 b	0.16 ab	0.033	ns	*
C22:5 n3	0.21	0.22	0.15	0.13	0.066	ns	ns
PUFA	14.26 c	14.76 bc	15.54 ab	16.13 a	0.779	***	ns

MS: sausages manufactured with boar back fat; MS+Y: sausages manufactured with boar back fat and inoculated with *D. hansenii*, GS: sausages manufactured with gilt back fat, GS+Y: sausages manufactured with gilt back fat and inoculated with *D. hansenii*.

¹ RMSE: Root mean square error.

² P_f: P value of the type of fat effect, P_y: P value of yeast effect, P_{fxy}: P value of interaction between type of fat and yeast effects. ***: P < 0.001; **: P < 0.01; *: P < 0.05; ns: P > 0.05. Different letters in the same row indicate significant differences among batches.

Table 2. Effect of back fat (from gilt or boar) and yeast inoculation on free fatty acids FFA (mg/100 g dm) in dry fermented sausages at 43 and 63 d.

Fatty acid	43d									63d								
	MS	MS+Y	GS	GS+Y	RMSE ¹	P _f ²	P _y	P _{fy}	MS	MS+Y	GS	GS+Y	RMSE	P _f	P _y	P _{fy}		
C12:0	1.67 b	1.75 b	1.98 ab	2.60 a	0.17	**	ns	ns	1.97 b	2.43 a	2.08 ab	2.32 ab	0.259	ns	*	ns		
C14:0	20.97 b	22.26 b	23.72 b	33.38 a	5.42	**	*	ns	25.46 b	31.38 a	27.17 ab	31.64 a	3.51	ns	**	ns		
C15:0	2.98	2.79	3.65	1.81	2.24	ns	ns	ns	2.81 a	1.70 ab	1.13 b	1.28 b	0.73	**	ns	*		
C16:0	303.55 b	319.58 b	331.61 b	459.42 a	71.33	**	*	ns	362.33 b	447.30 ab	398.23 ab	453.28 a	53.73	ns	**	ns		
C17:0	6.10	6.04	5.01	6.21	1.19	ns	ns	ns	6.91 ab	8.30 a	5.99 b	5.98 b	0.88	***	ns	ns		
C18:0	154.86 b	157.33 b	162.91 ab	204.35 a	26.96	*	ns	ns	171.43 b	207.19 a	196.05 ab	201.77 ab	21.69	ns	*	ns		
C20:0	1.27	1.27	1.33	1.65	0.25	ns	ns	ns	1.45	1.50	1.46	1.66	0.28	ns	ns	ns		
SFA	491.41 b	511.02 b	530.21 b	709.42 a	104.90	*	*	ns	572.37	699.80	632.11	697.94	79.76	ns	ns	ns		
C14:1	0.52	0.53	0.53	0.71	0.12	ns	ns	ns	0.59 b	0.73 a	0.57 b	0.62 ab	0.08	*	**	ns		
C16:1	45.79 b	48.84 b	54.63 b	76.05 a	11.95	**	*	ns	56.28 b	66.85 ab	60.16 ab	70.69 a	7.13	ns	**	ns		
C17:1	4.94	5.33	4.00	5.61	1.1	ns	ns	ns	6.09 ab	7.26 a	4.55 c	5.22 bc	0.86	***	*	ns		
C18:1	758.04 b	812.10 b	888.26 ab	1205.09 a	190.47	**	*	ns	899.98 b	1091.82 a	990.87 ab	1140.61 a	119.51	ns	**	ns		
C20:1 n9	19.97 b	22.65 b	26.06 ab	32.79 a	5.03	**	*	ns	25.31 b	31.49 a	28.80 ab	32.50 a	3.25	ns	**	ns		
MUFA	836.76 b	896.34 b	979.51 ab	1327.32 a	209.78	**	*	ns	996.87 b	1208.73 a	1091.34 ab	1256.84 a	131.13	ns	**	ns		
C18:2 n6c	375.42 b	412.99 b	531.74 ab	674.65 a	91.98	***	*	ns	465.29 c	603.00 b	616.17 ab	686.50 a	52.06	***	***	ns		
C18:3 n3	19.18 b	20.83 b	25.91 b	34.40 a	4.71	***	*	ns	25.33 c	31.64 a	31.92 a	35.47 a	3.01	***	**	ns		
C18:3 n6	0.63 ab	0.53 b	0.64 ab	0.74 a	0.10	*	ns	*	0.77	0.78	0.72	0.77	0.07	ns	ns	ns		
C20:2 n6	16.29 c	19.17 bc	26.26 ab	32.94 a	4.56	***	*	ns	21.44 c	28.25 b	30.46 ab	32.96 a	2.74	***	***	ns		
C20:3 n6	2.58	2.46	2.80	3.14	0.45	ns	ns	ns	3.36 ab	3.78 a	3.18 b	3.35 b	0.28	*	*	ns		
C20:4 n6	44.40	40.02	45.00	48.14	7.55	ns	ns	ns	58.19	67.08	58.71	61.06	5.68	ns	ns	ns		
C22:4 n6	8.26	7.61	8.47	9.78	1.62	ns	ns	ns	10.57 b	12.08 ab	10.67 ab	12.56 a	1.19	ns	**	ns		
C20:5 n3	3.16 ab	2.55 b	3.48 ab	3.54 a	0.59	*	ns	ns	4.40 a	3.98 ab	3.41 b	3.47 b	0.49	**	ns	ns		
C22:5 n3	17.07	15.49	16.21	18.52	3.71	ns	ns	ns	21.32 b	24.36 ab	20.84 b	25.63 a	2.51	ns	**	ns		
C22:6 n3	1.90	1.72	1.68	1.79	0.44	ns	ns	ns	2.47 ab	2.68 a	2.09 b	2.29 ab	0.30	ns	**	ns		
PUFA	489.64 b	524.28 b	662.19 ab	828.03 a	113.51	***	*	ns	613.66 b	778.84 a	778.55 a	864.05 a	64.04	***	***	ns		
TOTAL	1817.81 b	1931.64 b	2171.91 ab	2864.78 a	422.79	**	*	ns	2182.90 b	2687.37 a	2501.99 ab	2818.83 a	261.87	*	**	ns		

MS: sausages manufactured with boar back fat; MS+Y: sausages manufactured with boar back fat and inoculated with *D. hansenii*, GS: sausages manufactured with gilt back fat, GS+Y: sausages manufactured with gilt back fat and inoculated with *D. hansenii*.

¹ RMSE: Root mean square error.

² P_f: P value of the type of fat effect, P_y: P value of yeast effect, P_{fy}: P value of interaction between type of fat and yeast effects. ***: P < 0.001; **: P < 0.01; *: P < 0.05; ns: P > 0.05. Different letters in the same row indicate significant differences among batches.

Table 3. Effect of back fat (from gilt or boar) and yeast inoculation on most abundant and aromatic compounds (expressed as AU x 10⁻⁶) generated in dry fermented sausages after 43 and 63 d of processing.

Compound	43 d of processing				63 d of processing				RMSE ²	P _F ³	P _Y	P _{FxY}	MS	MS+Y	GS	GS+Y	RMSE	P _F	P _Y	P _{FxY}
	MS ¹	MS+Y	GS	GS+Y	MS	MS+Y	GS	GS+Y												
Aldehydes																				
2-Methylpropanal	0.48 c	0.63 bc	0.41 c	0.96 a	0.12 *	***	***	0.76 b	1.08 a	0.54 b	1.17 a	0.17 ns	***	*						
3-Methylbutanal (44) ⁴	0.49 a	0.20 c	0.41 ab	0.28 bc	0.11 ns	***	ns	1.15 a	0.59 b	0.86 ab	0.66 b	0.23 ns	***	ns						
Pentanal	45.13 a	4.51 b	42.21 a	6.05 b	13.15 ns	***	ns	24.25 a	0.82 b	4.23 b	0.97 b	3.65 ***	***	***						
Hexanal	343.06 a	59.26 b	298.24 a	88.74 b	83.40 ns	***	ns	238.16 a	14.26 c	112.17 b	17.01 c	45.35 **	***	**						
(E) 2-Hexenal (83)	0.18 a	0.02 b	0.15 ab	0.12 ab	0.08 ns	*	ns	0.10 a	0.02 b	0.04 b	0.01 b	0.03 *	***	*						
Heptanal (44)	2.34 a	0.69 b	1.64 ab	0.76 b	0.67 ns	***	ns	1.64 a	0.45 b	0.79 b	0.53 b	0.24 ***	***	***						
Octanal	11.44 a	2.68 b	7.00 ab	4.02 b	2.44 ns	***	*	5.42 a	1.39 c	3.63 b	2.21 bc	0.95 ns	***	**						
Nonanal	8.34 a	2.09 b	4.10 b	3.19 b	1.61 *	***	***	4.34 a	1.58 b	3.33 a	3.03 ab	0.85 ns	***	**						
(Z)2-Nonenal	0.77	0.39	0.37	0.64	0.28 ns	ns	ns	0.44	0.30	0.29	0.33	0.10 ns	ns	ns						
Ketones																				
2,3-Butanedione	7.07 a	0.80 b	11.07 a	1.26 b	3.15 ns	***	ns	10.25 b	1.05 c	21.99 a	1.64 c	3.26 ***	***	***						
2-Butanone	6.95 a	2.94 b	5.99 a	3.01 b	1.50 ns	***	ns	6.06 ab	5.24 ab	6.58 a	4.23 b	1.58 ns	*	ns						
3-Hydroxy-2-butanone	80.63 a	2.09 b	100.60 a	3.30 b	32.18 ns	***	ns	82.75 b	4.80 c	202.17 a	6.81 c	32.31 ***	***	***						
2-Heptanone (58)	1.08 a	0.41 c	0.76 b	0.67 bc	0.16 ns	***	***	0.94 a	0.53 b	0.80 a	0.50 b	0.14 ns	***	ns						
Acids																				
Acetic acid	177.90 a	75.03 b	205.93 a	91.41 b	31.38 ns	***	ns	218.91 b	99.12 c	306.15 a	107.44 c	40.88 **	***	*						
2-Methylpropanoic acid (43)	0.21 c	6.47 a	0.57 c	3.16 b	0.86 ***	***	***	3.74 c	8.58 a	5.18 bc	6.64 ab	2.00 ns	***	ns						
Butanoic acid (60)	7.79 a	4.09 c	8.75 a	5.91 b	0.81 ***	***	ns	7.60 b	4.64 c	9.30 a	5.98 c	0.95 ***	***	ns						
3-Methylbutanoic acid (60)	1.54 c	17.30 a	2.77 c	8.79 b	4.02 ns	***	*	11.35 b	19.83 a	11.25 b	14.96 ab	4.48 ns	**	ns						
Nitrogen compounds																				
2-Acetyl-1-pyrroline (43)	-	0.02	-	0.02	0.01 ns	***	ns	0.05 a	0.03 b	0.05 a	0.02 b	0.01 *	***	ns						
Sulphur compounds																				
Carbon disulfide	1.59 c	2.91 ab	2.63 bc	3.71 a	0.57 **	***	ns	3.12 c	7.50 a	2.28 c	5.02 b	1.03 ***	***	ns						
Dimethyl disulfide	0.15	0.19	0.09	0.21	0.07 ns	ns	ns	0.39	0.50	0.51	0.41	0.16 ns	ns	ns						
Methional	1.93 a	0.70 c	1.27 b	0.80 c	0.32 *	***	**	1.31 a	0.57 c	0.91 b	0.60 c	0.16 **	***	**						
Dimethyl trisulfide	0.12 a	0.08 ab	0.04 b	0.11 a	0.04 ns	ns	***	0.27	0.29	0.26	0.26	0.08 ns	ns	ns						
Alcohols																				

Ethanol	19.19 c	94.36 b	18.32 c	124.41 a	14.28 *	***	*	12.38 c	128.38 b	9.17 c	141.63 a	7.27 ns	***	*
3-Methyl-1-butanol	1.65 c	13.28 b	1.88 c	22.60 a	3.47 **	***	**	5.96 b	17.89 a	3.08 b	14.96 a	2.88 *	***	ns
2-Methyl-1-butanol	-	6.00 b	-	10.94 a	1.30 ***	***	***	3.03 b	8.35 a	-	8.36 a	1.99 ns	***	ns
1-Octen-3-ol (57)	2.07 a	0.17 b	1.77 ab	1.33 ab	0.93 ns	*	ns	1.38 a	0.23 b	0.46 b	0.14 b	0.30 ***	***	**
4-Methylphenol (107)	0.06	0.06	0.05	0.04	0.02 ns	ns	ns	0.06	0.06	0.06	0.06	0.02 ns	ns	ns
Phenylethyl alcohol (91)	0.01 b	0.03 a	-	0.01 b	0.01 ***	***	ns	0.01 a	0.02 a	0.004 b	0.02 ab	0.01 ns	**	ns
Esters														
Ethyl acetate	6.89 c	24.00 b	7.41 c	41.53 a	7.84 *	***	*	6.13 c	45.95 b	8.20 c	60.15 a	4.73 ***	***	**
Ethyl 2-methylpropanoate (43)	0.11 b	2.82 a	0.27 b	2.02 a	0.65 ns	***	ns	0.35 b	4.97 a	0.62 b	4.85 a	0.72 ns	***	ns
Ethyl butanoate	5.52 bc	8.91 b	4.42 c	17.20 a	2.02 ***	***	***	2.89 c	10.15 b	1.79 c	15.89 a	1.10 ***	***	***
Ethyl 2-hydroxypropanoate	1.50 c	5.20 b	1.62 c	9.02 a	1.49 **	***	**	2.14 c	9.19 b	2.28 c	11.64 a	1.17 *	***	*
Ethyl 2-methylbutanoate	0.21 c	9.03 a	0.28 c	5.52 b	1.51 *	***	*	0.36 b	13.00 a	0.34 b	11.26 a	1.67 ns	***	ns
Ethyl 3-methylbutanoate (88)	0.03 c	5.01 a	0.08 c	3.25 b	0.89 ns	***	*	0.30 b	7.87 a	0.30 b	7.16 a	1.13 ns	***	ns
Ethyl hexanoate (88)	0.13 b	0.26 b	0.16 b	0.66 a	0.08 ***	***	***	0.07 c	0.42 b	0.06 c	0.64 a	0.05 ***	***	***
Furans														
2-Pentylfuran	6.71 a	0.74 b	3.78 ab	0.83 b	1.92 ns	***	ns	2.95 a	0.87 b	1.29 b	0.93 b	0.47 ***	***	***
Aromatic hydrocarbons														
D-Limonene	1.46	1.01	0.71	1.22	0.53 ns	ns	ns	0.77 a	0.63 ab	0.52 b	0.73 a	0.11 ns	ns	**

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

¹ MS: sausages manufactured with boar back fat; MS+Y: sausages manufactured with boar back fat and inoculated with *D. hansenii*; GS: sausages manufactured with gilt back fat, GS+Y: sausages manufactured with gilt back fat and inoculated with *D. hansenii*.

² RMSE: Root mean square error.

³ P_f : P_f value of the type of fat effect, P_y : P value of yeast effect, P_{fy} : P value of interaction between type of fat and yeast effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; ns: $p > 0.05$. Different letters in the same row indicate significant differences among formulations.

⁴ Target ion used to quantify the compound when the peak was not completely resolved.

Table 4. Aroma compounds identified by olfactometry analysis in dry fermented sausages.

Compound	LRI ^a	LRI	Descriptor
	GC-O	GC-O std	
Aldehydes			
2-Methylpropanal	591	600	Fresh odour, butter, fruity
3-Methylbutanal	690	691	Fatty, hazelnut, muddy
Hexanal	835	836	Fresh cut grass
(E)2-hexenal	901	904	Fruity, oversweet
Heptanal	939	937	Grass, fresh, spicy odour
Nonanal	1050	1151	Talcum powder, sweet, cheesy
(Z) 2-Nonenal	1221	1222	Plastic, green odour
Ketones			
2-Heptanone	932	931	Fruity, sweet
Acids			
Acetic acid	700	700	Vinegar, acid
2-Methylpropanoic acid	873	876	Cheesy, rancid odour
3-Methylbutanoic acid	924	926	Roquefort cheese
Nitrogen compounds			
2-Acetyl-1-pyrroline	962	960	Savoury, snacks, toasted odour
Sulphur compounds			
Dimethyl disulfide	774	774	Rotten fruity or vegetable
Methional	966	964	Boiled potato or cauliflower
Dimethyl trisulfide	1008	1009	Unpleasant, sulphur, cabbage, onion
Alcohols			
1-Octen-3-ol	1024	1028	Mushroom
4-Methylphenol	1193	1190	Tyre, unpleasant
Phenylethyl alcohol	1195	1195	Floral, fresh odour
Esters			
Ethyl acetate	644	643	Floral, fruity
Ethyl 2-methylpropanoate	788	789	Sweet, fruity
Ethyl butanoate	825	825	Sweet, fruity
Ethyl 2-methylbutanoate	870	872	Strawberry, sweet
Ethyl 3-methylbutanoate	874	876	Syrup, sweet
Ethyl hexanoate	1029	1027	Pineapple, sweet
Aromatic hydrocarbons			
D-Limonene	1046	1048	Lemon
Unknown odours			
Unknown 1	569		Spicy, fresh odour
Unknown 3	757		Cheesy, fruity
Unknown 5	1032		Plastic, iron, metallic odour
Unknown 6	1179		Snacks, toasted odour
Unknown 7	1196		Boiled vegetables

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