Characterization of volatile compounds responsible for the aroma in naturally fermented sausages by GC-olfactometry

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Abstract

The objective of this study was to characterize naturally fermented dry sausages produced without the use of microbial starters and to determine which odour active compounds are responsible for their aroma. The traditional manufacture was responsible for different chemical characteristics and consumer's acceptance. The volatile compounds detected in the headspace comprised a complex mixture of volatile compounds derived from bacterial metabolism (mainly esterase activity of *Staphyloccoci*), spices and lipid auto-oxidation. The odour-active volatile compounds were identified using gas-chromatography coupled to olfactometry (GC-O) using the detection frequency method. The aroma profile was characterized by the presence of several compounds such as acetic acid, ethyl butanoate, hexanal, methional, 1-octen-3-ol, benzeneacetaldehyde and 4-methyl-phenol. However, naturally fermented sausages were also characterized by numerous esters, both ethyl and methyl esters, which impart a wide variety of fruity notes.

Keywords

Sausage, fermentation, aroma, volatile compounds, GC-olfactometry.

INTRODUCTION

The traditional manufacture methods of naturally fermented sausages and their singular organoleptic attributes distinguish them from others of the same category and make them highly appreciated by consumers. They are processed through a natural fermentation without the use of starter cultures and by drying in room chambers. A wide variety of traditional dry sausages are produced in the Mediterranean area, often at local or regional level (Talon and others 2008). In the east of Spain, small factories manufacture naturally fermented sausages “Embutido de Requena” (Requena, Valencia, Spain) through a natural fermentation process by indigenous bacteria and using low temperatures during processing (10-12 ºC).

Previous research has been done to characterize the microorganisms involved in the fermentation on naturally fermented sausages (Aymerich and others 2003; Talon and others 2008). Also, the changes of the lipid fraction that contribute to their final sensory quality have been studied (Navarro and others 2001). Nevertheless, few works have studied the volatile compounds present in naturally fermented sausages, (Croizet and others 1992; Mateo and Zumalacárregui 1996; Schmidt and Berger 1998a; Di Cagno and others 2009). Although in some cases it is no clear if the sausages were manufactured using a starter (Meynier and others 1999; Schmidt and Berger, 1998b; Ansorena and others 2001; Blank and others 2001).

Among the hundreds of volatile compounds identified in dry-fermented sausages only a limited number are present at a concentration higher than its threshold value contributing to the aroma. A few works have studied the aroma active compounds in dry fermented sausages (Stahnke, 1994; Schmidt and Berger, 1998b; Meynier and others 1999; Marco and others 2007; Olivares and others 2011). However, only Croizet and others (1992), Schmidt & Berger (1998a) and Söllner and Schieberle, (2009) have studied aroma compounds in naturally fermented sausages. Nevertheless, the relationship between aroma active compounds of naturally fermented sausages and their consumer acceptability has never been established. Therefore, the main objective

of this study was to study differences among naturally fermented Spanish dry sausages ("Embutido de Requena") and to determine which odour active compounds are responsible for their aroma.

MATERIALS AND METHODS

Materials

Naturally fermented dry sausages ("Embutido de Requena") from 10 different manufacturers (ER-1 to ER-10) were supplied by the "Consejo Regulador de la Indicación Geográfica Protegida Embutido de Requena" (Requena, Valencia, Spain). Artisan sausages were manufactured under the traditional specifications; no starter cultures were added and the drying process was carried out in room temperature chambers at low temperatures (approximately 10-12 ºC). The naturally fermented sausages were dried for approximately 1 month. The weight of naturally fermented sausages was 200-350 g and the diameter ranged from 30 to 40 mm. All the sausages were manufactured using lean pork meat, pork back fat, sodium chloride, nitrite, nitrate, sugars, dextrin, spices and different additives such as colorants (Ponceau 4R, carminic acid) and proteins (milk and soya). Three sausages from each "Salchichón de Requena" manufacturer (ER-1 to ER-10) were sliced, vacuum packed and frozen at -20 ºC for subsequent chemical, lipid and volatile analyses. Three sausages from each manufacturer were used for the consumer analysis. Results were expressed as the mean of three replicates in dry matter (dm).

Methods

Moisture, total lipids, protein, pH, TBARS, nitrate and nitrite and free fatty acids analyses. Moisture content was determined according to the official method for analysis of meat products by dehydration at 100 ºC until constant weight (BOE, 1979).

Total lipids were extracted from 5 g of minced sausage according to the method of Folch and others (1957), using dichloromethane:methanol (2:1) instead of

chloroform:methanol (2:1) as solvent due to its lower toxicity. The extracts were dried in a rotating vacuum evaporator and weighed to determine the total lipid quantity. Free fatty acids were analyzed as described Olivares and others (2011).

Nitrogen content was determined by the Kjeldahl method. The pH was measured as described by ISO 2917:1974 by introducing a pH-meter (HI 99163, Hanna Intruments Inc., Hoonsocket, USA) into a sausage : water mixture (1:1). Thiobarbituric acid reactive substances (TBARS) were determined according to Bruna and others (2001), using tricloroacetic acid instead of perchloric acid as solvent. The nitrate and nitrite content was determined using an enzymatic kit (Cat. No. 09050658, Roche, Palo Alto, USA) according to Arneth and Herold (1988).

Analysis of headspace volatile compounds. Extraction of headspace (HS) volatile compounds was done using a solid phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA) as described Olivares and others (2011). 3 g of minced sausage was weighted into a 10 mL headspace vial, and 0.75 mg of BHT was added. The vial was left for 1 h in a thermoblock (J.P., Selecta, Barcelona, Spain) at 37 ºC for equilibration. The CAR/PDMS fibre was then exposed to the headspace for 3 h while maintaining the sample at 37 ºC. For the identification of the volatile compounds, a gas chromatograph HP 7890A equipped with an HP 5975C mass selective detector (Hewlett Packard, Palo Alto, CA) using the same conditions as described Olivares and others (2011). The compounds were identified by comparison with mass spectra from the library database (Nist' 05), kovats retention index (Kovats, 1965) and by comparison with authentic standards. The standards used for the identification were all obtained from Fluka Chemie AG (Buchs, Switzerland) except (Z)-2-nonenal, (Z)-2-octenal, (Z,Z)-2,4-heptadienal, diacetyl, 2-methylpyrazine, benzeneacetaldehyde, methyl acetate, methyl 2-hydroxy-propanoate, and 4-methyl-phenol which were obtained from Aldrich (St. Louis, MO). Quantification was based on the total FID area. The results were

expressed as area units present in the headspace of sausage and comprised the mean of three replicates from each manufacturer.

The identification of aroma-active compounds was done by SPME-GC-Olfactometry (SPME-GC-O) as described Olivares and others (2011). The detection frequency method was used to estimate the aromatic impact of each volatile compound and two trained assessors evaluated the 10 naturally fermented sausages, therefore a total of 20 assessments were carried out.

Consumer sensory Evaluation. The acceptability of naturally fermented sausages was evaluated by 78 consumers. The analysis was carried out in a sensory laboratory equipped with individual booths (ISO 8589, 1988) as described Olivares and others (2011). The consumers were asked to evaluate each sausage based on appearance, aroma, hardness, juiciness, taste and overall quality using a 9-point hedonic scale. The analysis was done in two different sessions to avoid fatigue of the panelists due to the high number of samples, 5 samples in each session. Sensory evaluations were recorded by computer software using Compusense® *five* release 5.0 (Compusense Inc., Guelph, ON, Canada).

Statistical analysis. Data from chemical, free fatty acids, volatile and consumer analyses were analyzed by one-way analysis of variance (ANOVA) in order to determine differences among manufacturers. Differences between particular sample means were analyzed according to Fisher's least significant difference (LSD) test. Also, a Pearson correlation procedure was performed to evaluate any relationship between chemical and volatile compounds parameters. Furthermore, principal component analysis (PCA) was used to find the relationships among sausage manufacturers (ER-1 to ER-10) and chemical parameters. Statistical analysis was performed using the statistical software XLSTAT, 2009.4.03 (Addinsoft, Barcelona, Spain).

RESULTS AND DISCUSSION

Moisture, total lipids, protein, pH, TBARS, nitrate and nitrite and free fatty acids analyses.

Table 1 shows the composition and chemical characteristics of Requena naturally fermented sausages produced by different manufacturers. The water content of naturally fermented sausages ranged from 24.6 to 35.6 % while fat and protein contents ranged from 28.7 to 55.2 % and 32.3 to 53.7 % in dry matter (dm), respectively. Moisture, lipid and protein content are within the range expected for dry fermented sausages (Wirth, 1988), although the variability among sausages can be due to the raw materials used and also to differences in the traditional manufacturing practices.

In relation to pH, all the sausages were above pH 5.0 and all of them were between 5.3 and 6.05, except ER-4 (Table 1). Therefore “Salchichón de Requena” sausages can be considered as low-acid sausages (Aymerich and others 2003).

TBARS is used to evaluate the extent of the lipid oxidation process by measuring secondary lipid oxidation products. The TBARS values ranged from 0.6 to 2.8 mg malonaldehyde (MDA) per Kg dm and it is within the range expected for ripened sausages (Marco and others 2006). The lipid oxidation process that takes place during the ripening of dry sausages is essential for the development of typical aroma (Gandemer, 2002). Highest lipid oxidation values were detected in sausages that contained the highest amount of fat except for ER-4 sausage ($r = 0.865$, $p = 0.003$) in accordance to other studies where high oxidation values were related to high fat contents (Liaros and others 2009, Olivares and others 2011).

The nitrate content differed significantly between the sausages (Table 1). The concentrations ranged from 10.7 to 535.6 ppm (dm). Five naturally fermented sausages (corresponding to manufacturers ER-1, ER-2, ER-3, ER-4 and ER-5) showed high nitrate residual levels above the maximum residual limit (250 ppm). With respect to nitrite, only residual levels were found as other study reported (Meynier and others 1999).

In summary, the composition and chemical characteristics were different among naturally fermented sausages obtained from the different manufacturers, mainly due to differences in composition and the high nitrate residual level observed in several of them.

The total concentration of free fatty acids (FFA) in the naturally fermented sausages ranged from 1066.4 to 3211.1 mg/ 100 g dm (Table 2) which represented 2.5-8.0 % of the total lipid content. A similar FFA concentration was observed by Zanardi and others (2004) in dry fermented sausages. The FFA relationship detected in the sausages was MUFA>PUFA>SFA (Navarro and others 2001). However, Gandemer (2002) reported that the free fatty acid composition of back fat from industrial pigs was MUFA>SFA>PUFA. Therefore, it is supposed that the free fatty acid profile observed in this study was the consequence of the preferential release of the unsaturated fatty acids from the sausage lipids during ripening of sausages (Navarro and others 2001; Zanardi and others 2004).

In summary, the FFA profile was similar among sausages, although several of them showed lower FFA concentration. The differences in FFA among naturally fermented sausages are probably due to differences in the processing conditions among manufacturers such as grinding, stuffing, and the natural fermentation.

Analysis of headspace volatile compounds.

A total of 99 volatile compounds were identified by GC-MS in the HS of sausages and their structures were confirmed using authentic standards. However, several compounds could not be totally resolved and were quantified in the GC-FID as a mixture of volatile compounds (Table 3). Moreover, 4 compounds were not quantified in the sausages HS due to its low concentration (methanethiol, butanoic acid, 1-octen-3-ol and phenylethyl alcohol). The compounds detected were 27 esters, 19 aldehydes, 14 hydrocarbons, 9 alcohols, 9 acids, 8 ketones, 7 terpenes, 3 sulphur compounds, 2 pyrazines and 1 lactone. All of them have been previously identified in dry fermented sausages (Meynier

and others 1999; Schmidt and Berger, 1998; Ansorena and others 2001) except methyl benzeneacetate and methyl nonanoate.

The abundance of the volatile compounds is shown in Table 3, where the volatile compounds are grouped according to their possible origin: lipid auto-oxidation, bacterial metabolism (lipid β-oxidation, carbohydrate fermentation, amino acid degradation, and staphylococci esterase activity), spices and unknown origin (meat or food contaminants). However, several of the compounds listed can have more than one origin (Ordóñez and others 1999). With our extraction technique (SPME CAR/PDMS), the compounds extracted in higher amounts were those derived from lipid auto-oxidation, esterase activity of Staphylococci and spices (Figure 1). However, the proportion of volatile compounds obtained depends on the stationary phase of the SPME fibre employed and the extraction conditions used (time, temperature, etc). Therefore, the HS abundance and volatile compounds profile can not be compared with other studies which employed other extraction techniques such as dynamic headspace (Croizet and others 1992) and distillation techniques (Mateo & Zumalacárregui, 1996; Schmidt & Berger, 1998a) or other SPME extraction conditions (Di Cagno and others 2008; Spaziani and others 2009).

Lipid oxidation was responsible for the generation of volatile compounds mainly by auto-oxidation process as lipid auto-oxidation products comprised 14.5-34-4% of the total extracted area whilst lipid β-oxidation (produced mainly by Staphylococci as part of their metabolism) only generated 0.17-0.59% (data not shown). The manufacturers with the highest lipid derived compounds were ER-3, and ER-5 (Table 3). In this volatile group, the compounds that showed the highest abundance were hexanal, octanal, hexanoic acid, (Z,Z)-2,4-heptadienal and nonanal (Table 3). Using the same extraction technique Marco and others (2006) reported up to 45% of the extracted area of lipid auto-oxidation products in dry fermented sausages elaborated with starter culture that it is higher than the values obtained in naturally fermented sausages. However, the relative abundance obtained is in agreement with other naturally fermented sausages where the percentage

of lipid auto-oxidation volatile compounds was around 25% although using different extraction techniques (Mateo and Zumalacárregui, 1996; Di Cagno and others 2008; Spaziani and others 2009). In general, lipid derived volatile compounds represent one of the main groups in naturally fermented sausages although their relative importance is lower than in other fermented sausages. This fact could be due to the low temperatures applied during naturally fermented sausage processing, since lipid auto-oxidation is increased by temperature (Stahnke, 1995).

The volatiles generated from the carbohydrate fermentation represented 4.9-25.8% of the total extracted area (data not shown), although only ER-7 and ER-10 showed percentages above 20%. In this group, the compound extracted in the highest proportion was acetic acid (Table 3), although acetone was the most abundant compound in ER-3. It was observed an opposite relationship between acetic acid abundance and pH value, except in the manufacturer ER-4, since higher acetic acid abundance was related to lower pH (Pearson coefficient r=-0.7, p = 0.033). In general, the relative abundance of acetic acid in naturally fermented sausages is low compared to starter added sausages (Mateo & Zumalacárregui, 1996). Other authors also reported a low proportion of acetic acid in naturally fermented sausages (Schmidt & Berger, 1998a; Di Cagno and others 2008).

The volatile compounds originated from the amino acid degradation comprised 2.5-5.2 % of the total extracted volatile compounds (data not shown). The most abundant compounds in this group were 2-methyl butanoic acid, benzaldehyde, phenol and benzeneacetaldehyde (Table 3), which have been also detected in other naturally fermented sausages (Mateo and Zumalacárregui, 1996; Di Cagno and others 2008). The lowest abundance of this group was found in the sausages ER-5 and ER-8, mainly due to its lower abundance of benzaldehyde.

Esters were detected as the most abundant group of volatile compounds arising from microbial metabolism since they represented 17.6-44.1% of the total extracted area (Figure 1). Within the sausages, ER-6, ER-7 and ER-8 showed the highest ester

abundance. Two different types of ester compounds were identified; methyl and ethyl esters (Table 3). The methyl esters present in highest abundance were methyl acetate, methyl butanoate and methyl hexanoate (Table 3). Among the 12 methyl esters identified, 10 have been previously found in dry fermented sausages, except for methyl benzeneacetate and methyl nonanoate (Schmidt and Berger, 1998). The origin of methyl esters is not reported in the literature. Bruna and others (2001) remarked the ability of *Penicillium* strains to produce ethyl esters. However, Olivares and others (2011) also detected the presence of methyl esters in dry fermented sausages in which natamycin and potassium sorbate were added to prevent mould growth, therefore the generation of methyl esters could be attributed to *Staphyloccoci* (Montel and others 1998). With respect to ethyl esters, with our extraction technique, the most abundant ethyl esters in naturally fermented sausages were ethyl acetate and ethyl 2-methyl butanoate (Table 3).

The compounds derived from spices were mainly terpenes (Table 3) and represented 10.2-34.6% of the total extracted area (Figure 1) being ER-7 sausage the one with the lowest terpene abundance. In all the sausages, the most abundant compounds were β-myrcene, 3-carene and caryophyllene (Table 3), which are derived from pepper (Croizet and others 1992). The variability observed in the content of terpenes can be ascribed to the different amount and kind of spices added (Ordoñez and others 1999) as it was observed that some manufacturers used minced pepper while others used it as a whole grain. Spaziani and others (2009) detected terpenes as the most abundant class of aroma compounds (95% of the total extracted area) due to the spices added, mainly pepper and garlic, in natural Italian fermented sausages, although this was probably due to the extraction conditions employed. However, the high proportion of terpenes has also been described in other naturally fermented sausages in contrast to sausages with starter culture addition where other volatile compounds derived from biochemical reactions were dominant (Schmidt & Berger, 1998a; Di Cagno and others 2008). In Requena naturally fermented sausages, volatile compounds derived from spices

showed an important proportion of the extracted area, although they were not the main chemical class.

Aroma-active volatile compounds

The HS of naturally fermented sausages comprised a complex mixture of volatile compounds as described above. In order to determine the impact odorants responsible for their singular aroma the HS was analyzed by GC-O using the detection frequency (DF) method (Zellner and others 2008). A total of 42 odour-active regions were detected, although 11 of them could not be identified (Table 4, Figure 1), probably due to their low concentration but extremely low odour thresholds to be able to be detected by smell. All the aroma compounds identified have already been detected as odour active compounds in dry fermented sausages (Schmidt and Berger, 1998a; Meynier and others 1999; Marco and others 2007; Söllner and Schieberle, 2009) except for several esters (ethyl 2-OH-propionate, methyl 3-methyl butanoate, methyl benzoate and methyl benzeneacetate).

The detection frequency (DF) method assumes that the compounds detected more frequently have a greater relative importance. In this sense, only 6 compounds presented DF values higher than 16: acetic acid (vinegar), ethyl butanoate (strawberry, fruity), 1-octen-3-ol (mushroom), benzeneacetaldehyde (roses), 4-methyl phenol (stable) and one unknown compound defined as meat broth and savoury. In addition, sixteen compounds had DF values between 10 and 15: ethyl 2-methyl propanoate (strawberry), methyl 3-methyl butanoate (fruity), hexanal (fresh cut grass), butanoic acid (cheese), methional (cooked potato), (Z)-2-heptenal (unpleasant), ethyl hexanoate (flowery, sweet), α-terpinene (wood, metallic), caryophyllene (spicy, cloves) and 7 unknown compounds which contributed to the aroma with roasted nuts, toasted, woody and dissolvent notes. Within the volatile compounds with DF values below 10, it is worthy of note the high proportion of ester compounds both ethyl and methyl esters which impart a wide variety of fruity notes. Although methyl esters were more abundant than ethyl

esters (Table 3), most of the odour active esters were ethyl esters (Table 4) due to the higher thresholds of methyl esters than the ones of ethyl esters (Burdock, 2002). However, three methyl esters, methyl butanoate, methyl benzoate and methyl benzeneacetate, were identified as odour active compounds.

Many of the compounds which showed the highest HS abundance were detected as odour active (hexanal, octanal, acetic acid, benzeneacetaldehyde, ethyl acetate, methyl butanoate and ethyl 2-methyl butanoate, Table 3). However, the contribution of volatile compound to the aroma depends not only in HS abundance but also in perception threshold value. In this sense, several compounds were detected as important contributors due to their very low threshold values although they were extracted in low amounts ((Z)-2-heptenal, methional, 4-methyl phenol) or even not quantified (butanoic acid and 1-octen-3-ol) (Table 3).

Among the volatile compounds identified as odour-active in dry fermented sausages in previous studies, some of them have been selected as the most potent because they presented the highest DF values using detection frequency method or the highest dilution factors in dilution techniques (Zellner and others 2008). In this sense, the most potent odorants of naturally fermented sausages were acetic acid, ethyl butanoate, 1-octen-3-ol, benzeneacetaldehyde, 4-methyl phenol and methional also described as essential contributors to the aroma of dry fermented sausages (Stahnke, 1994; Schmidt and Berger, 1998a; Meynier and others 1999; Marco and others 2007; Söllner and Schieberle, 2009).

In naturally fermented sausages, only three studies have analyzed the odour-active compounds present. Firstly, Croizet and others (1992) indicated that sulphur compounds, acetic acid, diacetyl, linear and branched aldehydes were the main odorants in naturally fermented sausages, although these authors only described the aromas eluted from the GC but did not establish the most potent odorants. Secondly, Schmidt & Berger (1998a) reported the contribution of the spicy odour-active compounds derived from pepper and garlic (methylallysulphide, diallylsulphide,

diallyldisulphide and eugenol), but did not show the aroma compounds produced by biochemical reactions the latter being described as typical of the starter culture sausages. Finally, Söllner and Schieberle (2009) reported the compounds with the highest odour activity values (OAV) of Hungarian type salami as acetic acid, acetaldehyde, methional, phenylacetaldehyde, 2-metoxyphenol and 2-acetyl-1-pyrroline. This is in accordance with our results as acetic acid, bencenoacetaldehyde (phenyl acetaldehyde) and methional were also found as high aroma impact compounds in our naturally fermented sausages (table 4). On the other hand, compounds such as 2-metoxyphenol derived from the smoking process were not present in Requena sausages as they are not smoked. However, Requena naturally fermented sausages aroma was characterized by a high proportion of volatile compounds coming from biochemical pathways and to a lesser extent by spice-derived compounds.

Consumer sensory evaluation

In general, naturally fermented sausages were similar in sensory acceptance (Table 5). However, the ER-4 sausage was the less preferred by consumers in terms of hardness, juiciness, taste and overall acceptability ($p < 0.05$) and ER-5 sausage in terms of aroma ($p < 0.05$).

Principal Components Analysis

In order to establish which aroma compounds were responsible for the aroma of the naturally fermented sausages, a principal component analysis (PCA) was performed using the following parameters; lipolysis (FFA divided in SFA, MUFA and PUFA content), lipid oxidation (TBARS), aroma compounds abundance (only those compounds listed in Table 4). Results from PCA applied to mean scores of the parameters are summarized in Figure 2. The PCA showed that about 52.73% of the variability was explained by the two first principal components. Principal component 1

(PC 1) was the most important variable in terms of differences among samples as it accounted for 30.41% of the total variability.

PC1 was positively related to several ester compounds such as ethyl acetate, ethyl butanoate, ethyl 2-methyl propanoate, ethyl 2-hydroxy-propanoate, ethyl decanoate and methyl 3-methyl butanoate. In addition, PC1 was inversely related to octanal and the aldehydes (Z)-2-heptenal and 2-methylpropanal. On the other hand, principal component 2 (22.32%) was positively related to free fatty acids (SFA, MUFA and PUFA) and the aroma compounds acetic acid, α-terpinene, ethyl 2-methyl butanoate and ethyl hexanoate, and inversely to lipid oxidation value (TBARS), heptanal and hexanal.

CONCLUSIONS

Naturally fermented sausages from different manufacturers (Requena, Valencia, Spain) studied in this manuscript had different consumer acceptance and chemical characteristics. There was a wide variability among them that produced different consumer's acceptance probably due to the traditional manufacture process (i.e. gridding, stuffing and natural fermentation). Naturally fermented sausages were characterized by high free fatty acid content and high proportion of volatile compounds derived from lipid auto-oxidation, *Staphylococci* esterase activity, and spices. Among the volatile compounds, 31 were identified as odour active. The most odour active compounds were acetic acid, ethyl butanoate, hexanal, methional, 1-octen-3-ol, benzeneacetaldehyde and 4-methyl-phenol. However, the HS of the naturally fermented sausages was also characterized by numerous esters, both ethyl and methyl esters which impart a wide variety of fruity notes.

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Acknowledgements

Financial support from AGL 2009-08787 from MINECO (Spain), Prometeo 2012-001 (GVA) and FEDER funds are fully acknowledged. The predoctoral scholarship from GVA (Generalitat Valenciana, Spain) to A. Olivares is also acknowledged. The authors thank the Consejo Regulador de la Indicación Geográfica Protegida “Embutido de Requena” for the supplied samples, and are grateful to M. P. Valero for technical assistance.

Figure Legends

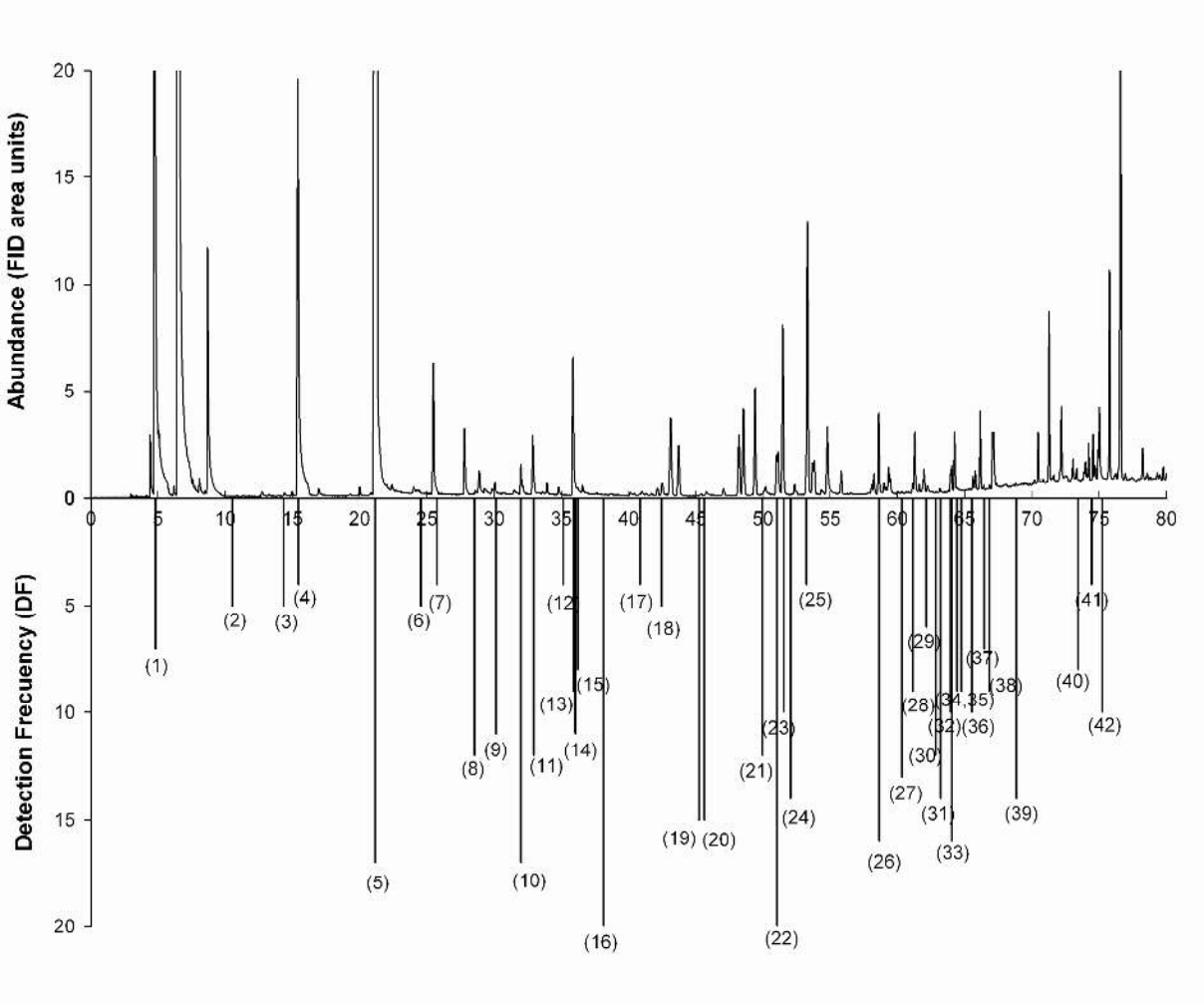


Figure 1. Aromagram of naturally fermented sausages. Numbers corresponds to the aroma described and indicated in table 4.

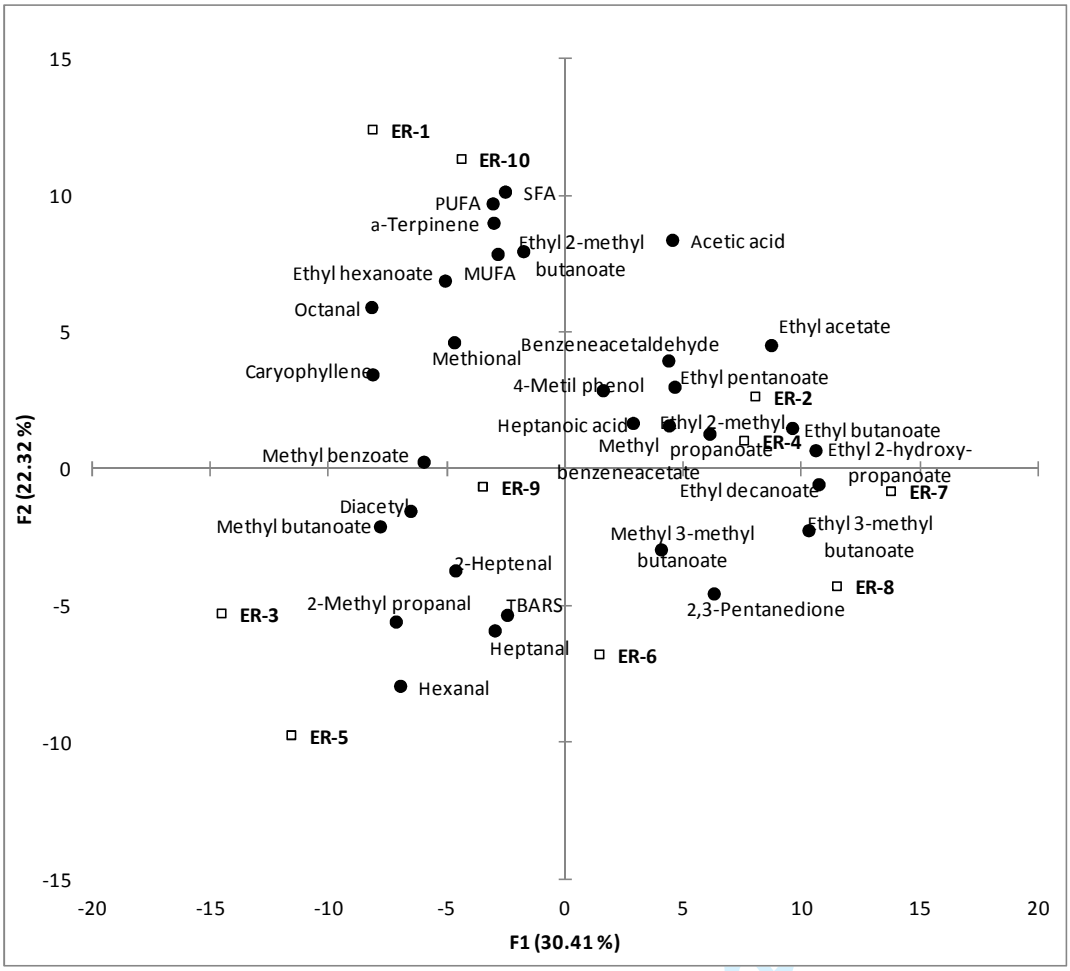


Figure 2. Loadings of the first two principal components (PC1-PC2) of the selected variables (●) and naturally fermented sausage samples (ER-1 to ER-10) (□). The selected variables were the aroma-active volatile compounds, TBARS and free fatty acids (SFA, MUFA, PUFA).

Table 1. Composition and chemical characteristics of naturally fermented sausages.

Samples	% water (g / 100g)	% fat (g / 100g dm)	% protein (g / 100g dm)	pH	TBARS (mg MDA*//kg dm)	Nitrate (ppm dm)	Nitrite (ppm dm)
ER-1	29.40 c	39.94 bc	43.36 c	5.56 b	1.34 c	206.72 bcd	10.47 bc
ER-2	33.28 b	33.10 d	53.73 a	5.55 b	1.01 cd	499.94 a	11.06 b
ER-3	28.12 cd	40.99 bc	37.71 d	6.00 a	1.28 c	535.65 a	12.47 a
ER-4	31.80 b	55.18 a	32.27 e	5.08 d	0.76 d	336.18 b	9.78 c
ER-5	24.66 e	44.75 b	36.96 d	6.00 a	2.08 b	269.43 bc	9.98 c
ER-6	27.06 d	51.45 a	35.64 d	6.05 a	2.78 a	10.73 e	11.14 b
ER-7	33.77 ab	42.32 bc	45.33 bc	5.36 c	1.29 c	110.89 de	8.66 d
ER-8	27.91 cd	42.48 bc	45.19 bc	5.38 c	1.04 cd	175.90 cd	8.79 d
ER-9	35.67 a	28.69 d	46.15 b	5.47 d	0.63 d	11.96 e	8.97 d
ER-10	33.45 ab	38.89 c	44.65 bc	5.53 d	0.98 cd	14.74 e	8.53 d
SEM**	0.784	1.697	0.927	0.032	0.173	52.280	0.251

*MDA: Malonaldehyde, [a-e]: Means that do not share any letter in the same column are significantly different (p <0.05). ** SEM: Standard error of the mean

Table 2. Free fatty acid (FFA) concentrations (mg/100 g dm) of naturally fermented sausages.

FFA	ER-1	ER-2	ER-3	ER-4	ER-5	ER-6	ER-7	ER-8	ER-9	ER-10	SEM**
C14:0	34.2 a	16.5 c	19.3 c	26.9 b	15.0 c	13.5 c	20.0 c	15.5 c	18.4 c	39.3 a	2.34
C16:0	407.1 a	249.8 cd	218.8 cd	268.5 c	223.3 cd	201.3 de	224.0 cd	159.2 e	195.5 de	341.5 b	20.11
C18:0	212.6 a	132.2 bc	98.9 d	141.1 b	93.2 de	97.2 de	104.4 cd	69.8 e	86.8 de	145.4 b	9.70
SFA*	653.9 a	398.5 cd	337.0 cde	436.5 bc	297.8 de	276.8 e	348.4 cde	244.5 e	300.6 de	526.2 b	36.64
C16:1	81.5 b	51.0 d	43.6 de	99.4 a	47.7 de	42.4 de	36.1 ef	36.8 ef	29.0 f	67.2 c	4.19
C18:1	1571.3 a	821.1 d	710.3 de	1347.0 b	646.6 def	688.9 de	571.5 efg	476.0 fg	387.5 g	1100.4 c	68.49
C20:1 *n9*	33.2 a	12.9 cd	12.1 cd	25.8 b	11.2 cd	17.0 c	12.9 cd	9.6 d	7.8 d	10.9 cd	2.28
MUFA	1685.9 a	885.0 c	766.0 cd	1472.1 a	698.7 cde	731.2 cde	620.6 def	522.4 ef	424.3 f	1178.4 b	74.31
C18:2 *n6*	708.7 a	346.8 cde	357.2 cde	428.9 c	319.9 def	307.7 def	367.3 cd	246.5 f	267.9 ef	587.1 b	32.46
C18:3 *n3*	45.3 a	17.3 cd	22.2 c	32.6 b	19.8 cd	19.1 cd	19.5 cd	14.9 d	15.3 d	32.8 b	2.10
C20:2 *n6*	25.6 a	10.4 ef	11.3 de	17.0 bc	11.7 de	13.8 cd	14.9 c	9.5 ef	7.7 f	19.0 b	1.07
C20:3 *n6*	10.6 a	8.2 bc	5.0 ef	6.2 de	4.8 ef	4.7 ef	6.9 cd	4.1 f	4.6 ef	8.8 b	0.55
C20:4 *n6*	55.0 a	43.1 b	30.4 c	29.1 cd	28.4 cd	29.6 cd	43.9 b	22.1 d	31.6 c	56.8 a	2.76
C22:4 *n6*	7.0 a	5.0 bc	4.3 cde	5.1 bc	3.9 de	4.4 cd	5.9 b	3.4 e	4.1 cde	7.4 a	0.34
C20:5 *n3*	1.5 a	1.1 bc	0.8 de	1.2 b	0.8 cde	0.6 e	1.1 bcd	0.6 e	0.7 e	1.2 b	0.11
C22:5 *n3*	14.1 a	8.0 bc	6.6 cd	12.7 a	6.2 cd	7.9 bc	9.8 b	5.9 d	7.7 cd	14.6 a	0.67
C22:6 *n3*	3.3 a	1.1 d	0.9 d	2.0 c	0.8 d	0.9 d	1.8 c	0.7 d	1.8 c	2.6 b	0.17
PUFA	871.3 a	441.0 cde	438.8 cde	534.7 c	396.3 def	388.8 def	471.2 cd	307.7 f	341.4 ef	730.3 b	39.38
Total	3211.1 a	1724.6 cd	1541.8 cd	2443.3 b	1848.7 c	1399.4 de	1440.1 cde	1074.6 e	1066.4 e	2434.9 b	151.39

* SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. [a-g]: Means that do not share any letter in a row are significantly different (p <0.05). ** SEM: Standard error of the mean.

Table 3. Volatile compounds in the headspace of naturally fermented sausages (GC-FID area units expressed as the mean of three replicates from each manufacturer).

KI	RI	Compound/origin	ER-1	ER-2	ER-3	ER-4	ER-5	ER-6	ER-7	ER-8	ER-9	ER-10	SEM
		Lipid auto-oxidation											
500	a	Pentane	1.57 cd	1.16 d	2.35 cd	3.42 cd	17.66 a	9.54 b	3.06 cd	1.63 cd	6.26 b	2.17 cd	1.61
621	a	Propanal	1.10 cd	0.00 d	17.30 a	0.63 d	6.80 b	4.01 bc	0.00 d	0.00 d	1.66 cd	0.00 d	1.05
600	a	Hexane	11.19 f	30.88 abc	16.23 ef	21.03 cdef	30.12 abcd	25.46 bcde	19.77 def	26.14 bcde	33.86 ab	36.98 a	3.52
614	a	1-propanol	16.41 c	10.39 d	25.41 b	8.53 de	5.73 e	10.41 d	23.86 b	18.11 c	49.31 a	7.67 de	1.11
625	a	Butanal	0.00 c	0.00 c	1.01 a	0.00 c	0.80 ab	0.50 b	0.00 c	0.00 c	0.00 c	0.00 c	0.08
700	a/a	2-Methylbutanal / heptane	0.00 c	3.38 bc	8.32 b	2.05 bc	8.85 b	6.83 bc	3.04 bc	1.62 bc	25.61 a	2.77 bc	2.81
735	a	Pentanal	8.84 bc	3.53 c	30.58 a	7.89 bc	25.50 a	12.48 b	9.65 bc	6.50 bc	7.96 bc	3.20 c	2.40
739	a/a	Ethyl propionate / 2,3-Pentanedione	2.56 c	7.83 ab	9.33 a	7.27 b	2.62 c	7.87 ab	9.17 ab	9.50 a	2.60 c	3.25 c	0.70
795	a	Propanoic acid	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d	18.58 b	0.00 d	30.48 a	0.00 d	4.85 c	0.52
800	a	Octane	2.10 c	3.29 bc	4.88 bc	4.80 bc	22.87 a	12.39 b	5.81 bc	3.50 bc	28.25 a	3.13 bc	3.21
836	a	Hexanal	82.20 c	47.65 c	323.38 a	95.04 bc	359.65 a	181.92 b	122.78 bc	96.14 bc	109.83 bc	62.48 c	31.93
904	a/a	2-Hexenal (Z) / Isopentyl acetate	0.00 h	1.77 g	3.73 e	2.50 efg	9.40 c	12.49 b	17.75 a	7.84 d	2.30 fg	3.30 ef	0.46
939	a	Heptanal	23.09 c	16.84 cd	40.62 b	40.38 b	67.90 a	38.77 b	55.65 a	4.54 d	38.54 b	16.09 cd	5.06
970	a/a	Methional / Pentanoic acid	10.85 a	1.60 d	5.20 c	0.88 d	5.91 c	5.53 c	9.11 b	1.75 d	9.63 ab	9.00 b	0.44
1011	a/a	2-Heptenal (Z)/ Butirolactone	5.67 bcd	0.00 d	13.30 b	3.19 cd	12.90 b	8.53 bc	7.70 bcd	3.66 cd	30.27 a	2.73 cd	2.85
1047	a	Octanal	3675.03 a	2552.22 bc	3705.44 a	1814.40 d	2061.78 cd	1565.85 d	672.33 e	1664.07 d	3174.85 ab	3796.34 a	212.11
1064	a	Hexanoic acid	219.36 a	139.44 c	177.86 b	72.49 e	96.80 d	59.43 e	76.55 de	139.00 c	173.64 b	193.64 b	8.09
1075	a	2,4-Heptadienal (Z,Z)	113.92 a	49.39 c	110.44 ab	29.60 d	14.85 d	17.62 d	47.06 c	24.14 d	94.55 b	119.99 a	5.51
1116	a	2-Octenal (Z)	3.49 d	0.37 e	6.87 b	2.30 de	6.73 bc	4.07 cd	3.62 d	2.29 de	26.41 a	1.52 de	0.93
1151	a	Nonanal	81.38 bc	49.46 cd	86.66 bc	40.33 d	112.53 b	110.36 b	187.32 a	116.76 b	177.02 a	57.08 cd	12.97
2159	a	Heptanoic acid	20.37 d	24.62 cd	27.98 c	258.87 a	11.30 e	10.77 e	28.25 c	12.61 e	10.47 e	50.44 b	2.28
1200	a	Dodecane	5.09 cd	4.47 de	2.62 ef	5.57 bcd	1.11 fg	1.86 fg	10.77 a	0.00 g	7.13 bc	7.27 b	0.72
1221	a	2-Nonenal (Z)	2.67 c	4.26 bc	13.27 b	3.13 c	3.47 bc	2.65 c	2.87 c	2.10 c	81.15 a	1.52 c	3.34
1253	a/a	Octanoic acid/decanal	64.83 ab	45.66 c	49.91 bc	1.53 d	57.38 bc	57.54 bc	41.07 c	80.48 a	49.59 bc	76.53 a	5.69
1326	a	2-Decenal (Z)	3.66 bc	1.86 de	2.57 cde	1.70 de	3.95 bc	3.23 bcd	4.60 ab	5.89 a	3.92 bc	1.01 e	0.61
1344	a	2-Undecanone	5.58 bcd	5.87 bc	3.57 cde	7.93 b	2.69 e	2.18 e	3.29 de	28.41 a	6.80 b	7.17 b	1.02
1440	b	Decanoic acid	35.21 c	31.53 cd	49.24 b	35.90 c	28.24 de	21.21 f	54.36 a	32.54 cd	23.66 ef	33.68 c	1.71
		Bacterial metabolism											
		Lipid β-oxidation											
937	a	2-Butanone	13.24 b	17.24 a	18.23 a	8.39 c	5.97 cd	4.20 d	18.08 a	9.25 c	0.00 e	7.90 c	1.14

730	a	2-Pentanone	0.00	e	0.00	e	20.46	a	0.50	de	2.83	b	0.63	d	0.52	de	0.47	de	1.60	c	0.55	de	1.96
931	a	2-Heptanone	1.55	d	0.00	d	14.28	b	2.32	d	13.09	bc	1.03	d	1.10	d	10.27	c	25.27	a	0.00	d	0.92
1142	a	2-Nonanone	21.10	cd	31.20	a	28.50	ab	8.50	f	17.50	de	21.63	cd	24.50	bc	0.00	g	13.75	e	28.55	ab	1.93
		Carbohydrate fermentation																					
528	a	Acetone	53.27	d	100.10	b	369.43	a	32.63	e	51.19	de	8.32	f	74.34	c	32.89	e	43.12	de	4.56	f	6.45
632	a	Diacetyl	0.00	e	2.33	cd	23.91	a	0.00	e	2.99	c	3.23	c	1.09	de	0.00	e	0.80	e	9.27	b	0.46
652	a	2-Butanol	3.52	f	7.05	cdef	7.44	cde	5.46	ef	9.34	bc	7.70	cde	6.02	def	8.71	bcd	11.44	ab	12.87	a	1.09
706	a	Acetic acid	1902.84	cd	1767.46	cd	273.76	f	1699.16	d	498.53	f	1309.27	e	2442.95	b	1396.48	e	1996.68	c	3292.36	a	98.41
		Amino acid catabolism																					
390	a	2-Methylpropanal	1.42	cd	1.37	d	2.20	bc	0.81	d	3.64	a	1.29	d	1.24	d	0.86	d	2.68	b	0.84	d	0.27
480	a	Benzene	0.55	de	0.40	e	1.12	b	1.35	ab	1.61	a	1.35	ab	0.78	cd	0.45	e	1.07	bc	1.09	b	0.11
583	a	2-Methyl-1-propanol	0.00	f	9.52	c	0.00	f	7.79	c	2.79	de	18.43	b	16.53	b	28.52	a	4.93	d	1.40	ef	0.83
691	a	3-Methylbutanal	4.48	def	8.68	cde	10.54	bc	2.45	f	9.06	cd	5.44	cdef	3.90	ef	0.77	f	14.41	b	23.48	a	1.74
774	a	Dimethyl disulfide	2.72	cd	13.80	bcd	173.41	a	0.00	d	18.83	bc	11.43	cd	27.98	b	0.00	d	13.14	bcd	179.30	a	5.56
853	a	2-Methylbutanoic acid	98.06	a	76.96	b	64.63	b	30.65	c	17.49	cde	10.92	de	9.86	e	30.21	cd	73.75	b	19.36	cde	6.53
1021	a	Benzaldehyde	466.30	ab	287.05	de	248.06	e	329.40	cd	136.10	f	158.85	f	99.54	f	149.44	f	534.83	a	411.87	bc	24.97
1103	a	Phenol	52.13	a	19.69	e	54.81	a	28.65	bcd	22.64	de	25.36	cde	4.90	f	22.03	e	29.85	bc	33.50	b	2.06
1111	a	Benzeneacetaldehyde	69.76	c	55.64	c	18.76	efg	25.26	def	10.92	fg	39.90	d	238.20	a	5.23	g	30.20	de	114.75	b	5.27
		Staphylococci esterase activity																					
449	a	Methyl acetate	513.20	b	345.55	d	185.61	e	432.85	c	218.83	e	654.42	a	635.17	a	314.88	d	326.88	d	287.86	d	22.97
542	a	Ethyl acetate	358.71	e	843.83	a	0.60	f	372.85	e	38.05	f	413.81	de	965.44	a	581.71	bc	513.90	cd	705.29	b	44.78
659	a	Methyl propanoate	26.84	cd	16.67	fg	41.53	b	30.31	c	44.80	ab	47.02	a	23.09	de	20.44	ef	22.54	de	14.88	g	1.71
744	a	Propyl acetate	8.84	a	0.52	cd	1.15	c	0.34	d	0.49	cd	0.64	cd	2.42	b	0.67	cd	2.59	b	0.72	cd	0.27
751	a	Methyl butanoate	994.51	b	618.56	cd	1024.70	ab	458.01	e	1127.54	a	711.16	c	538.49	de	470.45	e	324.12	f	522.46	de	42.91
789	a	Ethyl 2-methyl propanoate	10.67	b	2.95	f	0.00	g	4.15	ef	0.00	g	5.70	de	9.40	bc	13.50	a	7.30	cd	0.00	g	0.81
788	a/a	Toluene / Methyl 2-hydroxy-propanoate	131.50	cd	99.70	ef	79.08	f	166.55	b	153.70	bc	161.25	b	162.06	b	194.23	a	114.71	de	88.42	ef	9.16
803	a	Methyl 3-methyl butanoate	17.35	c	30.78	c	11.21	c	67.71	b	36.05	c	68.96	b	66.40	b	78.67	b	148.39	a	25.32	c	9.46
826	a	Ethyl butanoate	112.95	bc	211.14	a	1.30	f	90.16	cd	46.91	e	118.94	b	202.75	a	184.33	a	48.83	e	71.91	de	9.66
849	a	Methyl pentanoate	71.52	ab	39.21	d	77.41	a	43.25	d	76.73	a	61.59	bc	50.85	cd	16.80	e	44.96	d	63.32	abc	4.79
859	a/a	Ethyl 2-hydroxy-propanoate /2-methyl pirazine	22.83	c	44.37	b	0.00	e	46.68	b	4.32	e	20.04	cd	68.10	a	66.44	a	13.67	d	15.24	d	2.17
872	a	Ethyl 2-methylbutanoate	356.77	a	268.45	b	203.96	c	150.27	e	228.46	c	164.27	de	220.46	c	202.45	cd	161.59	e	281.67	b	13.29
876	a	Ethyl 3-methylbutanoate	0.00	e	19.68	c	0.00	e	22.21	c	3.75	de	18.91	c	34.30	b	44.57	a	4.73	de	8.53	d	2.75
924	a	Ethyl pentanoate	7.02	cd	12.23	b	0.96	f	6.30	d	4.02	e	8.62	c	15.13	a	0.00	f	11.23	b	7.07	cd	0.60
950	a	Methyl hexanoate	808.20	b	647.45	c	984.35	a	622.78	c	868.28	ab	656.59	c	668.40	c	849.79	b	548.38	c	577.06	c	42.80
1126	a	Ethyl heptanoate	3.04	bc	0.00	d	6.83	a	2.74	bc	7.73	a	3.72	b	3.83	b	2.10	bcd	1.90	bcd	1.13	cd	1.03

1156	a	Methyl octanoate	133.93	cd	92.78	de	132.78	cd	112.19	cde	104.67	cde	275.23	ab	293.81	a	233.49	b	146.16	c	77.06	e	14.16
1226	a	Ethyl octanoate	87.75	d	157.65	b	2.96	g	2.87	g	41.79	f	134.07	c	155.56	b	242.68	a	5.00	g	61.80	e	7.05
1237	a	Methyl benzeneacetate	0.00	d	202.73	a	1.93	cd	98.47	b	1.47	cd	0.36	d	3.16	cd	1.78	cd	5.60	c	2.13	cd	1.65
1255	a	Methyl nonanoate	9.60	e	34.93	d	45.39	c	76.40	a	12.48	e	3.67	f	34.94	d	13.34	e	38.37	d	58.03	b	1.45
1323	a	Ethyl nonanoate	2.95	cd	4.13	bc	0.25	d	6.70	ab	1.50	cd	1.96	cd	6.11	b	5.97	b	9.70	a	1.28	d	0.95
1354	a	Methyl decanoate	11.59	cd	10.79	cd	15.11	c	30.41	a	14.35	c	28.74	a	33.36	a	21.89	b	9.07	d	7.84	d	1.61
1421	a	Ethyl decanoate	28.18	d	87.02	b	8.85	f	59.57	c	9.58	ef	64.61	c	107.90	a	90.13	b	23.23	def	26.46	de	5.36
		Derived from spices																					
945	a	α-Pinene	239.98	b	214.62	b	159.36	c	44.57	d	35.09	d	26.25	d	10.38	d	29.79	d	165.10	c	351.95	a	13.51
3003	a	β-Myrcene	2312.18	a	1183.03	d	1978.21	b	814.07	e	493.89	f	379.68	f	153.79	g	572.49	f	1635.03	c	2070.80	ab	74.14
1026	a/a	Ethyl hexanoate / 3-carene	1303.84	a	764.95	c	979.80	b	687.97	c	423.65	d	362.09	d	301.01	d	690.27	c	859.67	bc	772.38	c	66.70
1037	a/a/a	α-terpinene / trimethyl pyrazine / octanone	61.99	b	37.71	c	37.43	c	18.66	d	7.40	d	9.38	d	35.19	c	14.10	d	64.67	b	79.23	a	4.79
1052	a	p-Cymene	443.25	a	422.48	a	450.98	a	271.96	b	174.12	cd	107.64	e	130.12	de	199.28	c	428.40	a	328.60	b	19.72
1051	a	Limonene	468.55	a	361.33	b	301.88	b	177.32	c	180.54	c	178.40	c	98.25	c	85.02	c	520.54	a	295.87	b	33.64
1148	a/a	Linalol / Methyl benzoate	71.26	bc	77.71	ab	85.74	a	3.34	g	65.91	cd	52.87	ef	46.38	f	50.86	ef	50.21	ef	59.55	de	3.22
1255	a	Terpineol	13.09	ab	15.73	a	14.07	ab	13.80	ab	7.98	cd	5.81	d	5.88	d	6.52	cd	10.24	bc	15.13	a	1.40
1480	a	Cariophyllene	840.01	ab	214.21	e	710.74	bc	431.83	d	600.82	c	751.77	bc	213.05	e	429.92	d	820.18	ab	985.71	a	57.07
		Unknown origin, meat or food contaminants																					
883	a	Ethyl benzene	2.40	c	9.47	b	3.23	c	2.38	c	1.72	c	2.71	c	2.03	c	0.74	c	32.58	a	0.65	c	1.41
893	a	p-xylene	14.04	cd	24.66	ab	15.67	c	24.84	a	12.82	cd	16.74	bc	13.63	cd	7.16	d	30.31	a	17.59	bc	2.44
918	a	o-xylene	4.06	d	9.17	cd	6.57	cd	11.66	c	22.01	b	43.90	a	6.02	cd	8.57	cd	26.30	b	3.41	d	2.15
920	a	Styrene	4.77	b	11.40	a	5.41	b	6.39	b	5.78	b	5.37	b	1.87	c	5.12	b	5.10	b	3.96	bc	0.97
1190	a	4-methyl phenol	3.67	c	3.47	c	3.73	c	13.70	a	3.20	c	3.03	c	3.23	c	4.23	c	8.37	b	7.66	b	0.48
1330	a	Tridecane	3.23	d	2.10	e	2.13	e	7.74	a	1.70	e	2.17	e	3.08	d	6.52	b	4.73	c	3.32	d	0.29
1390	a	Caprolactame	1.07	bc	0.38	e	2.26	a	0.50	de	2.67	a	0.87	cde	1.04	cd	0.49	de	0.58	cde	1.62	b	0.19
1400	a	Tetradecane	13.30	g	10.87	g	114.67	b	41.57	efg	78.43	cd	91.24	bc	22.07	fg	52.63	def	195.37	a	64.83	cde	10.51

[A]: Kovats indices calculated for DB-624 capillary column (J&W Scientific: 60 m, 0.32 mm i.d., 1.8 µm film thickness) installed on a gas chromatograph equipped with FID detector. [a-h]: Means that do not share any letter in a row are significantly different (p <0.05). * SEM: Standard error of the mean. [C] Reliability of identification: a, mass spectrum and retention time identical with an authentic standard; b, tentative identification by mass spectrum.

Table 4. Odour-active compounds identified in the HS of dry fermented sausages.

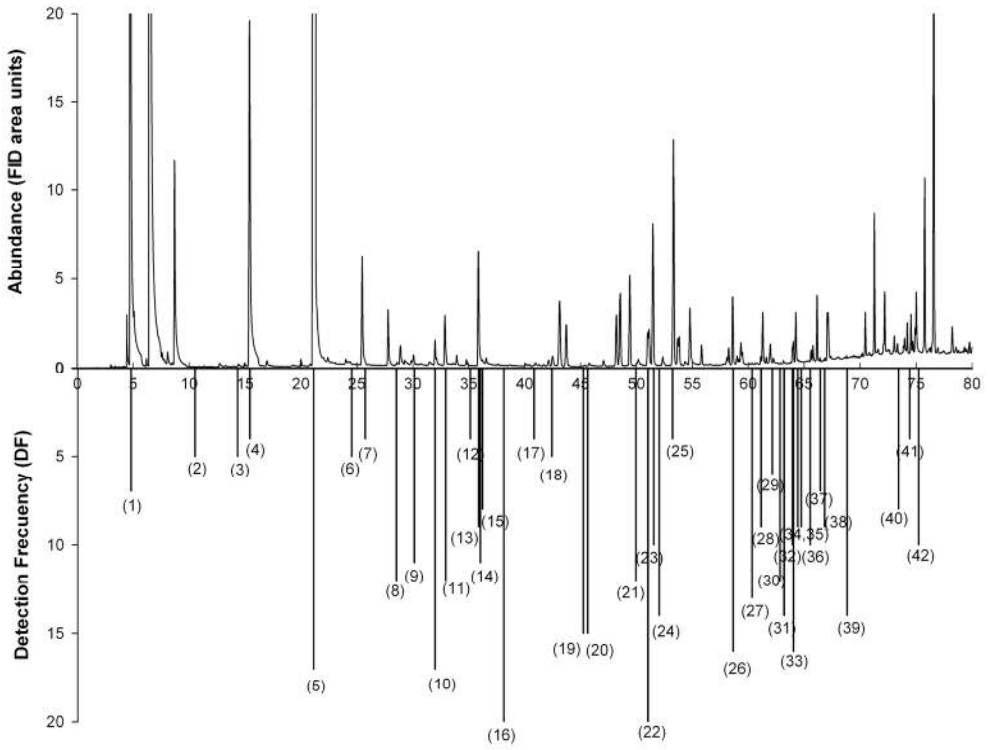
N*	IK**	Compound***	GC-O descriptor	DF****
1	472	Methanethiol	Rotten	7
2	590	2-Methylpropanal	Fresh, cologne	5
3	630	Diacetyl	Butter, caramel	5
4	642	Ethyl acetate	Vegetal	4
5	702	Acetic acid	Vinegar	17
6	740	2,3-Pentanedione	Sweet, milky	5
7	753	Methyl butanoate	Fruity	4
8	784	Ethyl 2-methylpropanoate	Strawberry	12
9	802	Methyl 3-methylbutanoate	Fruity	11
10	824	Ethyl butanoate	Strawberry, fruity	17
11	835	Hexanal	Fresh cut grass	12
12	861	Ethyl 2-hydroxy-propanoate	Fresh	4
13	870	Ethyl 2-methylbutanoate	Fruity	9
14	874	Ethyl 3-methylbutanoate	Fruity, floral	8
15	872	Butanoic acid	Cheese	11
16	898	Unknown 1	Meat broth, savory	20
17	924	Ethyl pentanoate	Floral, fresh, fruity	4
18	937	Heptanal	Herbal	5
19	963	Unknown 2	Roasted nuts, toasted	15
20	966	Methional	Cooked potato	15
21	1007	2-Heptenal (Z)	Unpleasant, cabbage	12
22	1020	1-Octen-3-ol	Mushroom	20
23	1025	Ethyl hexanoate	Flowery, sweet	10
24	1031	α-Terpinene	wood, metallic	14
25	1045	Octanal	Citric	4
26	1109	Benzeneacetaldehyde	Roses	16
27	1135	Unknown 3	Toasted, old wood, burnt hair	13
28	1147	Methyl benzoate	Fruity, sweet, waxy, metallic	9
29	1162	Heptanoic acid	Fresh, herbal	6
30	1172	Unknown 4	Woody, pine	12
31	1178	Unknown 5	Roasted nuts, toasted	14
32	1189	Unknown 6	Mustiness, woody	10
33	1190	4-Methyl phenol	Stable	16
34	1195	Phenylethyl alcohol	Roses	9
35	1201	Unknown 7	Mustiness	9
36	1222	Unknown 8	Wood, toasted, herbal	15
37	1236	Methyl benzeneacetate	Caramel, sweet, toffee	7
38	1245	Unknown 9	Toasted, coffee	9
39	1286	Unknown 10	Glue, dissolvent	14
40	1400	Unknown 11	Wood, pistachio, spicy	8
41	1424	Ethyl decanoate	Fruity	4
42	1443	Caryophyllene	Spicy, cloves	10

* N: number of compound as shown in figure 1. **IK: Kovats index calculated for DB-624 capillary column (J&W Scientific 60m x 0.32 mm i.d., film thickness 1.8 µm) installed on a gas chromatograph equipped with a flame ionization detector (FID) and a sniffing port. *** Identification by mass spectrum, coincidence with the LRI of an authentic standard and by coincidence of the assessors's descriptors with those described in the Fenaroli's handbook of flavour ingredients (Burdock, 2002).
**** DF Detection frequency value.

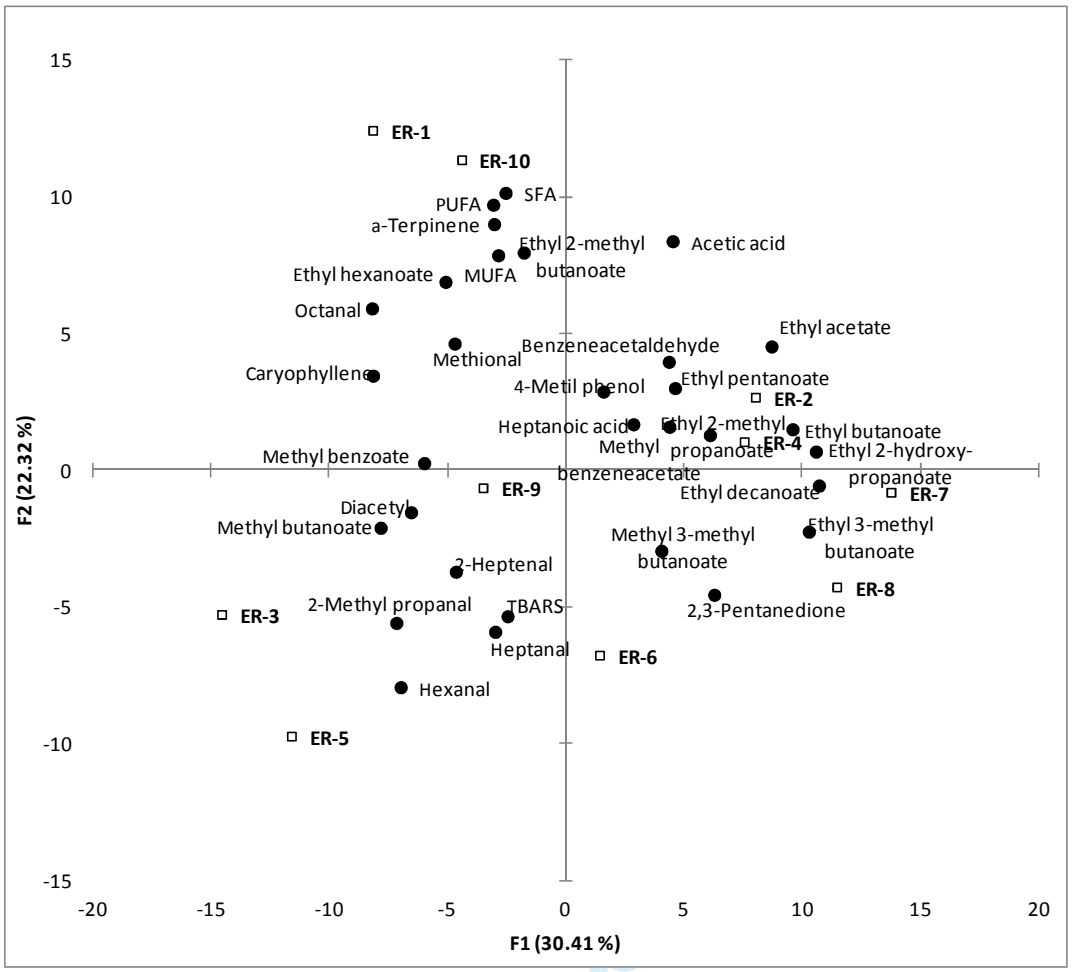
Table 5. Consumer analysis (hedonic test) of naturally fermented (ER) sausages.

Samples	Appearance[1]	Aroma	Toughness/hardness	Juiciness	Taste	Overall quality
ER-1	6.24 ab	6.35 a	6.38 ab	6.57 a	6.62 a	6.42 a
ER-2	6.11 ab	6.46 a	6.24 ab	6.24 a	5.91 c	6.1 a
ER-3	6.44 a	6.24 a	6.53 a	6.44 a	6.19 abc	6.16 a
ER-4	4.94 c	6.39 a	4.97 c	5.57 b	5.3 d	5.01 b
ER-5	5.84 b	5.67 b	6.44 ab	6.44 a	6.34 abc	6.25 a
ER-6	6.28 ab	6.48 a	5.97 b	6.38 a	6.08 bc	6.08 a
ER-7	6.42 a	6.23 a	6.23 ab	6.55 a	6.57 ab	6.37 a
ER-8	6.15 ab	6.29 a	6.34 ab	6.38 a	5.84 c	6.01 a
ER-9	6.2 ab	6.3 a	6.06 ab	6.33 a	6.26 abc	6.12 a
ER-10	5.23 c	6.14 a	6.53 a	6.61 a	6.24 abc	6.42 a
SEM	0.175	0.156	0.179	0.16	0.185	0.176

[a-d]: Means that do not share any letter in a column are significantly different (p <0.05). * SEM: Standard error of the mean. [1] Scale used: 9-point hedonic.



Aromagram of naturally fermented sausages. Numbers corresponds to the aroma described and indicated in table 4.
186x156mm (300 x 300 DPI)


15
10
5
0
-5
-10
-15
-20
-15
-10
-5
0
5
10
15
20
F2 (22.32 %)
F1 (30.41 %)
ER-1
ER-10
PUFA
SFA
a-Terpinene
Ethyl 2-methyl butanoate
Acetic acid
Ethyl hexanoate
MUFA
Octanal
Ethyl acetate
Methional
Benzeneacetaldehyde
Caryophyllene
4-Metil phenol
Ethyl pentanoate
ER-2
Heptanoic acid
Ethyl 2-methyl propanoate
ER-4
Ethyl butanoate
Methyl benzoate
Ethyl 2-hydroxy-propanoate
Methyl benzeneacetate
ER-9
Ethyl decanoate
ER-7
Diacetyl
Methyl butanoate
Methyl 3-methyl butanoate
Ethyl 3-methyl butanoate
2-Heptenal
ER-8
ER-3
2-Methyl propanal
TBARS
2,3-Pentanedione
Heptanal
ER-6
Hexanal
ER-5