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VOLATILE COMPOUNDS ANALYSIS OF DACIA SAUSAGE, A TRADITIONAL ROMANIAN DRY-CURED SAUSAGE

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Three batches of Dacia sausage were produced as follows: one without starter culture and two with a mix of starter cultures. Volatiles were extracted by a purge-and-trap method and analyzed by gas chromatographic/mass spectrometry. Approximately 43 compounds were identified. The substances identified belong to: aldehydes, alcohols, ketones, hydrocarbons, esters, acids, furans. Results indicated that the most abundant class of chemical substances in flavor at the end of the ripening process was esters, followed by aldehydes, hydrocarbures and terpenes. This could only indicate the high microbial esterification activity that took place in the batches. It was possible to differentiate between the three sausages applying a discriminant analysis.

Keywords: Dacia dry sausage, volatile profile, starter culture

Introduction

Romanian dry sausages are usually fermented sausages that undergo a more or less prolonged period of drying-ripening before consumption. Some of them are also smoked, before the drying-ripening process.

The most representative Romanian dry sausage is *Sibiu sausage*, made from lean pork and fat, spices and other additives. The specificity of *Sibiu sausage* is represented by the long drying-ripening period and the mould covering of the product. There are various recipes of Romanian dry sausages, the mixes used in the manufacturing of these sausages including: pork, beef, beef and pork, beef and mutton, pork fat, salt, garlic, pepper and other spices and additives. A specific spice added is allspice, which can be found also in *Sibiu sausage*. The mixes are stuffed into natural or artificial casings, optionally smoked, then they undergo a drying-ripening process.

Dacia sausage is a Romanian dry sausage produced from lean pork, fat, salt, garlic, pepper, allspice, $NaNO_3$ and sucrose. The mix is stuffed into natural casings and then smoked, dried and ripened. Traditionally it was produced without starter

culture addition. It is a smoked dry sausage with a specific flavor due to its manufacturing process.

Selected starter cultures could improve the fermentation process as well as the safety and standardization of fermented sausages. The most important microorganisms used as starter cultures in meat fermentation are Lactic Acid Bacteria (LAB) and Micrococcaceae.

Microorganisms, in particular catalase-positive cocci, may influence the aroma and taste of fermented sausages by transforming compounds originating from (non-microbial) lipid and protein degradation into compounds which add to the desired aroma of the sausages. For example, the levels of 2-alkanones and esters were higher in salami prepared with a mixed starter than in the control sample (Schmidt and Berger, 1998), and the levels were positively correlated with salami odor and levels of *Staphylococcus xylosus* (Stahnke, 1995). Different staphylococci vary in their effects (Berdague et al., 1993).

The application of autochthonous *Staphylococcus equorum* and *S. succinus* in combination with *Lactobacillus sakei* contributes to the preservation of the original sensory qualities of traditional fermented dry sausages (Talon et al., 2008).

Generally, different starter cultures will result in various sensory characteristics of dry sausages. The flavor of the final product has a strong influence on the consumer acceptability. It is an important characteristic for the overall quality of the sausage. There are some factors that influence the volatile profile of the final product such as: bacterial metabolism, lipid oxidation, addition of spices, curing agents, fermentation stage (Marco et al., 2008).

The aim of this study was to determine if the use of starter cultures can influence the generation of flavor compounds during fermentation and the ripening period of Dacia sausage. We also wanted to see if there are any sensorial differences between dry sausages fermented by different starter cultures.

Material and methods

Sampling

In order to carry out this study, three batches of Dacia sausage were produced in triplicate as follows: one without starter culture addition (batch A) further referred to as control batch, one with a mix of *L.sakei* CECT 5964 and *S.equorum* SA25 (batch B) and one with a mix of *L.sakei* CECT 5964, *S.equorum* SA25 and *L.acidophilus* CECT 903 (batch C). Each batch followed the same recipe consisting of: lean pork (80%), fat (20%), salt (2.65%), $NaNO_3$ (0.075%), garlic (0.035%), sucrose (0.180%), white pepper (0.260%), allspices (0.075%). Starter cultures were added at the dose of 5×10^6 CFU/g. From each batch of sausage, samples at 7, 14 and 28 days of ripening were taken.

Volatile compounds analysis

Samples were lyophilized (Labconco Corp., Freezone, Kans., U.S.A.) during 48 h at -40 ºC and then ground in a domestic blender, after which, 10 g weighed, put

into a dynamic headspace vial for further analyses. The volatile compounds were extracted and concentrated in a purge-and-trap concentrator coupled with a cryofocusing module (Teledyne Tekmar, Mason, Ohio, U.S.A.).

Dynamic headspace volatile concentration

Lyophilized samples were transferred into headspaced vials and concentrated in a purge-and-trap concentrator (Stratum, Teledyne Tekmar, Mason) equipped with a cryofocusing module which was connected to an autosampler (Solatek 72 Multimatrix Vial Autosampler, Teledyne Tekmar, Mason). Each sample was maintained at 80 ºC for 1 min and then flushed with helium at a flow rate of 40 mL/min for 22 min (Purriños et al., 2011). The volatile compounds were adsorbed on a Tenax Trap (Supelco, Bellafonte, Pa., U.S.A.). Volatile compounds were thermally desorbed from the Tenax trap at 225 ºC for 4 min with a helium flow rate of 300 mL/min. The desorbed compounds were cryofocused at -30 ºC using liquid nitrogen at the entrance of a DB-624 capillary column (J&W Scientific, Folsom, Calif., U.S.A.).

Gas chromatography/mass spectrometry

A gas chromatograph 6890N (Agilent Technologies Spain, S.L., Madrid, Spain) equipped with mass detector 5973N (Agilent Technologies Spain, S.L.) was used with a DB-624 capillary column (J&W scientific: 30 m × 0.25 mm i.d., 1.4-μm film thickness). Each sample was injected in split mode (1:20). Helium was used as a carrier gas with a linear velocity of 36 cm/s. The temperature program used was as follows: 40 ºC maintained for 2 min and then raised from 40 to 100 ºC at 3 ºC/min, then from 100 to 180 ºC at 5 ºC/min, and from 180 to 250 ºC at 9 ºC/min with a final holding time of 5 min; the total run time being 50.78 min. Injector and detector temperatures were set at 220 and 260 ºC, respectively. The mass spectra were obtained using the mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1576 V, and collecting data at a rate of 6.34 scans/s over the m/z 40 to 300. Compounds were identified by comparing their mass spectra with those contained in the NIST05 (Natl. Inst. of Standards and Technology, Gaithersburg, Md.,U.S.A.) library, standard alkanes (C_5 to C_{14}) (for calculating Kovats indices, Supelco 44585-U, Bellefonte) and by matching their retention indices with those reported in literature. Nine samples were analyzed in triplicate. Results were reported as relative abundance expressed as total area counts (AU × 106) (Purriños et al., 2011).

Data analysis

The data was subjected to an analysis of variance (ANOVA) using the General Linear Model procedure of the computer programme Statistica 8.0 for Windows (Statsoft Inc., Tulsa, OK, USA) to determine the overall effect of starter cultures and ripening time.

Differences in the volatile compounds profile of the three sausages were determined using the statistical techniques of the canonical discriminant analysis. Furthermore a canonical discriminant analysis was performed in order to observe

the differences in the volatile compounds of different sausages and a 0.01 tolerance was established.

Results and discussion

Volatile analysis

A total of 38 compounds were identified in batch A (control), while 41 compounds were identified in batch B and 44 in batch C.

These compounds include a total of: 13 aldehydes, 13 hydrocarbons, 8 esters, 7 terpenes, 2 alchools, 3 ketones, 1 acid and 1 amine. They were not present in all batches and in all sampling stages. The results of individual volatile compounds are summarized in Table 1, as well as the levels of significance for the volatile compounds.

Several compounds were absent or in existed in very low quantities in the initial mass. The phenomenon could be attributed to the strong lipolytical activities which, as we assume, began after four days of ripening. Long and medium chain fatty acids were detected in high quantities in the three batches. These fatty acids may act as a source of compounds with certain effect on the aroma, but they are not directly involved in the dry cured products aroma (Árboles and Juliá, 1992).

Although oleic acid is the predominant acid in sausages, acetic acid was the only acid determined in the three batches. The levels of acetic acid found in this study were about 25% in batch A, 19% in batch B and 10% in batch C from the total area of peaks. The presence of acetic acid was also reported by other authors (Berger et al., 1990; Schmidt and Berger, 1998) in sausages, while Meynier et al. (1999) did not detect it.

In general, with regard to volatile compounds derived from lipid oxidation, we can see that aldehydes were present in higher amounts in the inoculated batches than in the control sausage. Aldehydes are probably the most interesting class of volatile compounds from a flavor and odour generating point of view. These compounds represented 5% of the total peak area at the end of the process. The main aldehyde was hexanal, which offers a green grass odour (Stahnke, 1994), being produced during the oxidative degradation of unsaturated fatty acids (Frankel, 1980; Grosch, 1982). High concentrations of hexanal signal flavor deterioration in meat products often resulting in a rancid aroma (Pham et al., 2008; Ramirez and Cava, 2007). In addition, heptanal and butanal were also detected. There was one branched short-chain aldehyde 3-methyl-butanal, which is produced during the degradation of leucine through a non enzymatic Strecker reaction (Berdague et al., 1993; Barbieri et al., 1992) or by microorganisms (Bailey et al., 1992). This compound was associated with a ripened aroma in cured meat products (Sondergaard and Stahnke, 2002). It can be transformed into the corresponding alcohol, acid and even ester, as it is of great importance in the final flavor of the products.

Table 1. Percentage of area relative to the total peak area of the identified peaks of volatile compounds of the three sausages during ripening (mean-chromatographic area ± standard deviation) and level of significance as influenced by time and starter culture

	Control (days)				Batch B (days)				Batch C (days)				
	7	14	28	D	7	14	28	D	7	14	28	D	B
Compounds													
Terpenes													
alpha pinene	ND	ND	0.92±0.15	***	ND	0.46±0.46	0.84±0.22	*	1.05±0.10	0.54±0.28	1.17±0.20	-	-
beta pinene	ND	ND	0.59±0.29	***	ND	0.2±0.2	0.17±0.17	-	0.41±0.05	0.32±0.16	1.09±0.21	*	-
beta myrcene	ND	ND	0.44±0.05	***	ND	ND	0.18±0.18	-	ND	ND	0.44±0.24	-	-
alpha phellandrene	ND	0.15±0.15	ND	-	0.14±0.14	0.19±0.19	ND	-	ND	ND	0.14±0.14	-	-
3-carene	ND	ND	4.27±0.33	***	7.57±1.53	ND	3.76±1.20	**	7.90±0.88	ND	4.69±0.43	***	-
D-limonene	0.27±0.27	0.35±0.35	2.53±0.33	**	ND	ND	1.92±0.39	**	ND	ND	2.71±0.48	***	-
Caryophylene	ND	ND	ND	-	ND	ND	ND	-	ND	ND	ND	-	-
Total	0.27±0.27	0.51±0.30	8.74±0.64	***	7.72±1.40	0.84±0.84	6.88±1.57	*	9.37±0.90	0.86±0.43	10.26±1.62	**	-
Amines													
Methenamine	ND	ND	ND	-	ND	ND	ND	-	ND	ND	ND	-	-
Furans													
furan.3-methyl	0.60±0.30	1.52±0.26	0.20±0.10	*	1.67±0.92	0.71±0.36	0.12±0.12	-	1.19±0.25	0.80±0.12	0.09±0.09	**	-
Aldehydes													
pentanal	ND	ND	ND	-	0.29±0.29	0.99±0.72	0.85±0.46	-	13.44±2.74	1.01±0.71	0.57±0.57	**	-
hexanal	3.61±0.93	5.18±2.14	1.70±0.44	-	27.28±2.07	7.63±1.48	2.27±0.28	***	20.41±2.10	5.35±1.37	2.56±0.33	***	-
2-hexenal	ND	ND	ND	-	ND	ND	ND	-	0.20±0.20	ND	ND	-	-
heptanal	ND	ND	0.25±0.24	-	1.187±0.67	0.48±0.46	0.52±0.52	-	0.44±0.25	0.56±0.56	0.45±0.45	-	*
2-heptenal	ND	ND	0.13±0.13	-	ND	0.39±0.39	0.39±0.39	-	0.39±0.23	0.50±0.50	0.21±0.21	-	*
nonanal	ND	ND	ND	-	ND	ND	ND	-	0.19±0.19	ND	ND	-	-
2-furancarboxaldehyde	0.26±0.26	ND	0.13±0.13	-	ND	ND	0.29±0.29	-	ND	0.41±0.41	ND	-	-
2.4-heptadienal	ND	ND	ND	-	ND	ND	ND	-	0.15±0.15	ND	ND	-	-
butanal 3-methyl	0.84±0.42	ND	1.15±0.31	-	0.87±0.47	0.7±0.41	1.41±0.24	-	1.35±0.74	1.21±0.36	0.87±0.48	-	-
furfural	3.22±0.75	2.06±0.65	ND	*	3.24±0.24	2.42±0.25	0.89±0.45	**	3.41±0.63	3.39±0.77	0.89±0.18	*	-
2-n-butylacrolein	ND	ND	ND	-	ND	ND	ND	-	ND	ND	ND	-	-
Total	8.53±1.69	8.76±2.23	3.57±0.84	-	34.54±2.29	13.31±2.50	6.74±2.25	***	41.21±4.12	13.27±1.74	5.68±1.51	***	-

Hydrocarbons												
Heptane, 2.2.4.6.6-pentamethyl	ND	4.45±2.19	4.47±1.39	-	0.73±0.73	1.40±1.21	8.38±1.21	**	0.82±0.82	0.43±0.43	2.28±0.87	-	-
Decane, 2.2.4-trimethyl	ND	0.71±0.35	ND	-	ND	ND	ND	-	1.11±0.35	0.18±0.18	ND	*	-
2.2.7.7-tetramethyloctane	3.32±3.09	3.41±1.61	0.17±0.17	-	ND	ND	0.43±0.22	-	0.94±0.94	1.28±1.28	ND	-	-
Nonane, 5-methyl	0.88±0.88	2.21±1.13	ND	-	1.08±0.13	ND	ND	***	1.24±0.26	1.13±0.06	ND	*	-
Nonane, 3.7-dimethyl	ND	ND	ND	-	2.72±0.25	0.85±0.43	ND	**	3.06±0.63	2.69±0.34	ND	**	-
Undecane, 4.8-dimethyl	1.85±0.25	0.91±0.47	ND	-	1.89±0.35	1.26±0.63	ND	*	1.42±0.23	1.62±0.33	ND	*	-
Undecane, 4-methyl	1.27±0.22	1.69±0.84	ND	-	2.43±0.13	ND	ND	***	2.51±0.42	2.15±0.58	ND	*	-
Heptane, 4-ethyl-2.2.6.6-tetramethyl	ND	ND	ND	-	ND	ND	ND	-	ND	ND	ND	-	-
octane 2.6-dimethyl	1.24±0.34	0.14±0.14	ND	*	0.44±0.28	ND	ND	-	ND	ND	ND	-	-
octane	0.23±0.23	0.15±0.15	0.33±0.33	-	0.77±0.32	0.52±0.52	0.64±0.64	-	1.03±0.58	0.10±0.10	ND	-	-
heptane	ND	ND	0.12±0.12	-	0.61±0.47	ND	0.15±0.15	-	ND	0.39±0.22	0.29±0.29	-	-
hexane	2.67±1.85	3.90±0.50	1.75±0.38	-	2.77±0.62	5.01±0.32	1.92±0.55	-	2.16±0.49	2.69±0.19	1.61±0.27	-	-
benzene 1-methyl-4-(1-methylethyl)	ND	ND	0.79±0.16	***	ND	1.42±0.71	0.52±0.26	-	ND	ND	0.84±0.13	***	-
Total	19.55±2.25	17.61±5.39	7.65±2.04	*	13.48±0.98	10.48±1.22	12.06±1.66	-	14.33±1.28	12.7±2.25	5.05±1.32	*	-
Alcohols													-
1-pentanol	ND	ND	ND	-	0.29±0.29	ND	0.32±0.32	-	0.53±0.53	ND	ND	-	-
1-octanol, 2-butyl	ND	ND	ND	-	ND	ND	ND	-	ND	ND	ND	-	-
Total	ND	ND	ND	-	0.29±0.29	0	0.32±0.32	-	0.53±0.53	ND	ND	-	-
Ketones													
2.3-butanedione	2.67±1.39	3.21±0.54	1.04±0.88	-	2.96±2.00	1.5±0.80	0.43±0.43	-	2.88±1.28	2.17±0.70	0.33±0.23	-	-
3-hexanone	ND	ND	1.44±0.97	-	ND	ND	ND	-	ND	ND	ND	-	-
2-cyclopenten-1-one. 2-methyl	0.06±0.06	ND	1.06±0.08	***	ND	0.23±0.23	0.93±0.14	*	ND	0.34±0.34	0.91±0.54	-	-
Total	2.67±1.39	3.21±0.54	2.49±1.24	-	2.96±2.00	1.5±0.80	0.43±0.43	-	2.88±1.28	2.17±0.70	0.33±0.23	-	-

Acids															
Acetic acid	ND	ND	28.78±3.12	***	15.11±1.41	43.38±4.81	19.04±1.10	**	24.55±4.53	36.99±4.12	10.43±2.45	**	-		
Esters															
Acetic acid, methyl ester	27.18±3.61	32.99±4.02	31.11±3.61	-	23.11±1.26	31.24±4.62	33.86±5.71	-	7.74±1.55	28.80±2.46	40.78±2.87	***	-		
butanoic acid, methyl ester	1.06±0.64	0.40±0.40	5.28±0.74	**	0.20±0.20	0.73±0.38	6.59±0.83	** *	0.30±0.30	0.76±0.38	13.17±1.24	***	-		
pentanoic acid, methyl ester	ND	ND	1.29±0.23	***	ND	ND	0.50±0.26	-	ND	ND	3.39±1.04	*	-		
hexanoic acid, methyl ester	0.17±0.17	0.14±0.14	2.07±1.15	-	0.46±0.46	0.38±0.19	1.75±0.24	**	0.82±0.82	0.58±0.32	2.99±0.81	-	-		
Octanoic acid. methyl ester	ND	ND	0.28±0.28	-	ND	ND	ND	-	ND	ND	0.62±0.33	-	-		
Malonic acid. bis (2-trimethylsilyl ethyl ester)	1.04±0.33	0.51±0.05	0.24±0.13	-	0.25±0.13	0.15±0.15	0.20±0.20	-	0.25±0.04	0.24±0.12	ND	-	-		
3-hidroximandelic acid. ethyl ester di-TMS	2.73±0.69	1.75±0.16	1.69±0.53	-	0.81±0.15	ND	1.25±0.17	**	0.66±0.07	0.96±0.13	1.43±0.62	-	-		
methyl propionate	ND	0.51±0.26	5.45±1.38	**	1.02±0.31	0.37±0.19	4.43±0.44	** *	1.37±0.35	1.20±0.11	2.56±0.50	-	-		
Total	32.21±4.56	36.34±4.65	47.45±0.77	-	25.87±0.99	32.50±5.50	48.61±6.94	-	11.16±0.66	32.56±2.46	64.98±5.49	***	-		
Alkanes															
heptane	ND	ND	0.34±0.34	-	0.61±0.47	ND	0.16±0.16	-	ND	0.39±0.22	0.29±0.29	-	-		
octane	0.23±0.23	0.15±0.15	0.33±0.33	-	0.77±0.32	0.18±0.18	0.31±0.31	-	1.03±0.58	0.58±0.58	ND	-	-		
hexane	2.01±1.04	3.90±0.50	1.75±0.38	-	2.77±0.62	5.01±0.32	1.92±0.55	*	2.16±0.49	2.69±0.19	1.61±0.27	-	-		
Total	2.31±1.19	4.05±0.46	3.50±1.01	-	4.16±0.51	5.44±0.15	3.33±0.99	-	3.19±0.21	4.01±0.60	2.82±0.71	-	-		

D: significantly different values as influenced by time; B: significantly different values as influenced by starter culture. ***P<0.001; **P<0.01; *P<0.05; - no significant difference
ND: not detected

Alcohols are mainly generated as reaction products of lipid oxidation (Shahidiet al., 1986). In this study, two alcohols were identified, but only one, 1-pentanol, appeared in one of the inoculated sausages till the end of the ripening period. 1-pentanol was also detected by Garcia-Esteban et al. (2004), Muriel et al. (2004) and Ramirez and Cava (2007) in other raw cured meat products. In general, primary unbranched alcohols produce grassy or woody aromas and make an overall contribution to the odour (Garcia et al., 1991). Because of their low odour threshold, they are important contributors to the aroma of these products (Sabio et al., 1998).

Alkanes were found in small amounts and their origin is probably the oxidation of branched fatty acids present in animal tissues or the unsaponifiable fraction of vegetable animal feed. The same is true for ketones, found in small amounts and deriving from lipid oxidation. Three ketones were identified, 3-hexanone, 2-cyclopenten-1-one, 2-methyl and 2,3-butandione. Diacetyl imparts a buttery odour, having a characteristic sweet odour and a low sensory threshold and, according to Stahnke (1995), is of great importance to the final aroma.

Several hydrocarbons have been identified in the volatile fraction of the dry sausages produced. Aliphatic hydrocarbons increased during processing but the percentage of these compounds in the total chromatographic area decreased as processing time elapsed. Aliphatic hydrocarbons do not contribute significantly to the aroma of dry-cured meat products because of their high threshold value (Ramirez and Cava, 2007). The percentage of these compounds was significantly higher in batch B. Low weight hydrocarbons, such as octane, hexane and heptanes, were found in all batches.

Terpenes represented between 8 and 10% of the total peak area, having their origin in the use of spices in sausages. Molecules such as α-pinene, α-phellandrene, 3-carene have been detected by Ekundayo et al. (1998) in pepper and β-caryophyllene, limonene were isolated in paprika (Guadayol et al., 1997). Some of them were described as fruity, floral and fresh rather than spicy (Meynier et al., 1999). In dry-cured ham, as suggested by Sabio et al. (1998), the presence of limonene is associated with the pig's diet. In this study, the most important terpenes found were 3-carene and D-limonene. These two products, as the other five in this work, are thought to have their origin in the use of black pepper.

Esters have been indicated as important volatiles in fermented sausages (Edwards et al., 1999; Stahnke, 1994) and they are also present in high quantities in our study. They originate from alcohols and carboxylic acids by the action of microorganisms (Sabio et al., 1998). They represented about 47% in the control batch and in batch B, reaching 61% in batch C (Fig.1).

It is well documented that many strains of lactic acid bacteria used as starter cultures are able to produce esters (Hosono et al., 1974; Liu et al., 1998). They have low odour threshold values and impart fruity notes (Stahnke, 1994), being associated, together with branched aldehydes, to ripened flavor in cured meat products (Hierro et al., 2004). Methyl-esters were the main esters produced in the three batches and their production may be attributed to microorganisms if we take

into consideration the high microbial counts in the present study (~11 log c.f.u). The main ester identified in this study was the metylic ester of acetic acid, which represented about 40% of the total peak area.

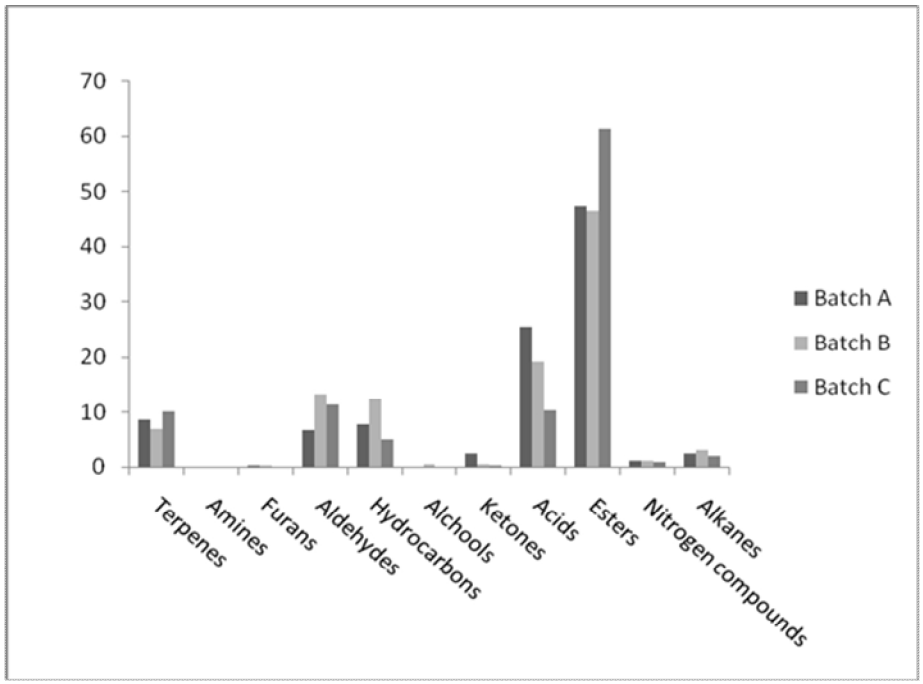

Figure 1. Classes of volatile compounds present in the three batches at the end of ripening

When a canonic discriminant analysis was performed, it was possible to differentiate the three sausages on the basis of their content (%) in volatile compounds: heptane, 2,2,4,6,6 pentamethyl, total ketones, acetic acid, acetic acid methyl ester, methyl propionate.

The following discriminant functions of classification were obtained:

F_1= - 0.17898[heptane, 2,2,4,6,6 pentamethyl] – 3.55647[total ketones] – 0.78699[acetic acid] – 0.08676[acetic acid, methyl ester] – 3.60355[methyl propionate]

F_2= - 1.05620[heptane, 2,2,4,6,6 pentamethyl] + 0.81878[total ketones] – 0.38770[acetic acid] + 0.08571[acetic acid, methyl ester] + 0.71335[methyl propionate]

When the results from function F_1 were plotted against the results obtained from function F_2 on axes of coordinates for each volatile compound, a good discrimination between the volatile compounds of the three batches was observed (Fig. 2).

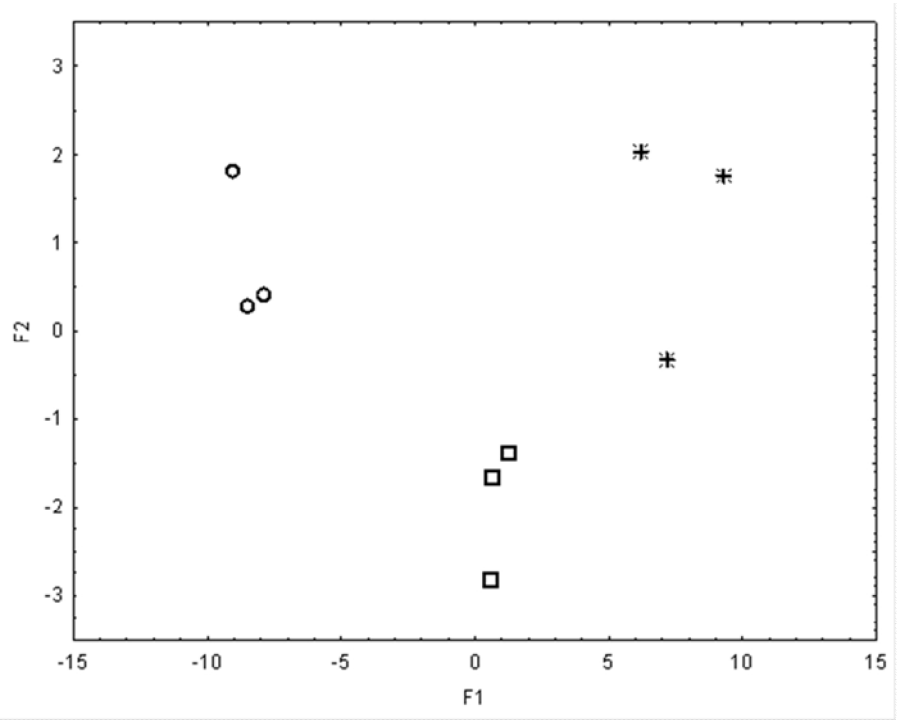

Figure 2. Plot of the samples on the axes representing the values of the two discriminating functions

Conclusions

The sensory diversity of dry-cured sausages is related to specific manufacturing practices. The use of starter cultures led to changes in sensory properties and even more so in the profile of volatile compounds. Most of the volatiles present in this study were associated with microorganisms. The esters produced added fruity and floral notes to the aroma of the sample sausages. Lipid oxidation provided significant amounts of volatiles, the same as the spices used.

We assume that the differences in the aroma of different dry-cured sausages are mainly related to the starters composition and the utilization of spices.

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