TITLE: Analysis of volatile compounds by GC-MS of a dry fermented sausage: chorizo de Pamplona.

AUTHORS: Diana Ansorena, Olga Gimeno, Iciar Astiasarán,* and José Bello.

Departamento de Bromatología, Tecnología de Alimentos y Toxicología, Facultad de Farmacia, Universidad de Navarra, 31080-Pamplona, Spain. Phone: 948-425600 (Ext 6264). Fax 948-425649. E-Mail: iastiasa@unav.es

*To whom correspondence should be addressed

ABSTRACT

The profile of volatile compounds of a typical Spanish dry fermented sausage, chorizo de Pamplona, has been analyzed by GC-MS, using a simultaneous distillation-extraction (SDE) system. Qualitative and quantitative differences were found in the volatile profiles obtained in the five analyzed commercial brands. One hundred and ninety three different substances were isolated, the group of acids being the most important from a quantitative point of view in all brands, accounting at least for the 60% of the total area. Aldehydes, basically from lipid oxidation, contributed between 7.72% and 13.97% to the total amount. Acids and aldehydes were the chemical families that showed the lowest variability among brands. In contrast, esters showed the highest coefficient of variation among brands (111%), followed by phenols (82%) and terpenes (76%). The variability observed in these three families could be attributed respectively to the different starter cultures, smoking process and spices employed in their production. Butylated hydroxytoluene (added as an antioxidant, E-321) was the third most abundant compound in 3 of the 5 brands.

Keywords: Chorizo de Pamplona; volatile profile; SDE extraction

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INTRODUCTION

Chorizo de Pamplona is a traditional dry fermented sausage with a relevant interest in the meat industry of Spain. Some studies about the chemical and microbiologycal proceses that take place during the ripening (Gorospe et al., 1989; Astiasarán et al., 1990a; Astiasarán et al., 1900b; Gimeno et al., 2000), which are the origin of the sensorial properties of this product, have been carried out.

One important sensory property which has been hardly studied is the "flavour" of the product. Many studies have been carried out to study the flavour of different cured meat products. Volatile components of dry cured ham have been studied by Berdagué et al. (1991), Buscailhon et al. (1993), Hinrichsen et al. (1995) and Dirinck et al. (1997). Aroma components from dried sausages fermented with *Staphylococcus xylosus* were described by Stahnke (1994, 1995, 1999a, 1999b) and Johansson et al. (1994) followed the evolution of volatile compounds during the ripening of a fermented sausage elaborated with *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter cultures. Berger et al. (1990) identified 68 different compounds in dry salami whereas Croizet et al. (1992) identified 53 in dry saucisson. Meynier et al. (1999) studied the relationship between the volatile compounds isolated from commercial Milano salami and their olfactory properties. In chorizo, Mateo et al. (1996b) published results of the analysis of both traditional and industrial chorizo and detected 126 peaks among which 115 were identified. In this paper, these authors found that acetic acid, allyl-1-thiol and phenol were the major components for their products.

Most of these studies have been carried out using a dynamic headspace system of extraction of the volatile compounds. However, a simultaneous steam distillation-extraction (SDE) with a modified Likens-Nickerson apparatus for the isolation of the compounds was used by Mateo et al. (1996b) and Dirinck et al. (1997). The latter authors compared the SDE extraction and the dynamic headspace isolation to study

differences between northern and southern European cured hams. They concluded that because reliable semi-quantitative data were aimed at, SDE extraction should be preferred over dynamic headspace isolation. In this work, in order to make a "total volatile analysis" of the product, the Likens-Nickerson system of isolation of these compounds was chosen.

The objective of the present study was to analyze the profile of the volatile compounds of different commercial brands of chorizo de Pamplona to increase the knowledge of the substances responsible for its characteristic flavour.

MATERIAL AND METHODS

Four sausages of five different commercial brands of chorizo de Pamplona were randomly purchased at different supermarkets of the city (Pamplona, Navarra, Spain). They were frozen and stored at -20ºC until the analysis. The common ingredients used in this type of products are: lean pork meat, pork back fat, salt, sugars (dextrin, lactose, dextrose), sodium ascorbate, nitrites and/ or nitrates, red pepper, spices, colorants (Ponceau 4R E-124) and exogenous proteins (powdered milk or sodium caseinate). The technological process include: mincing of lean pork meat and pork back fat in a cutter to a particle size reduction of 3mm, mixing with the other ingredients in a vacuum kneading machine, stuffing into artificial casings and a further fermentation, smoking and drying process, during a period of about 30 days.

Likens-Nickerson extraction

A total of 25 g of frozen sausage was ground and placed in a 250 ml flask with 100 ml of water. A second flask with 5 ml of dichloromethane and 150µg of dodecane (internal standard-i.s.) was also attached to a modified Likens-Nickerson apparatus. A total of 5 ml of dichloromethane was also added to fill the apparatus solvent return loop. Sample mixture and solvent were heated to 70ºC and boiling Tª respectively, mantaining these

conditions during 2h. After cooling to ambient temperature, the extract of dichloromethane was collected and dried over anhydrous Na_2SO_4.

Analysis of volatile compounds

The volatile compounds were analyzed using a HP 6890 GC System (Hewlett-Packard) coupled to a 5973 Mass Selective Detector (Hewlett-Packard, Carretera Nacional-VI, Km. 18, 400, 28230 Las Rozas, Madrid, Spain). A total of 1 µl of the extract was injected into the GC, equipped with a capillary column (30 m x 250 µm i.d. x 0.25 µm film thickness HP-5MS, Las Rozas, Madrid, Spain). Carrier gas was He (1ml/min) and the chromatographic conditions were as follows: initial oven temperature was maintained at 40ºC for 10 min, and subsequently programmed from 40ºC to 120ºC at a rate of 3ºC/min and at a rate of 10ºC/min from 120ºC to 250ºC where it was held for another 5min. Injector T^a: 250ºC ; Mass range: 30-350 amu ; Solvent Delay: 4 min. ; Electron impact at 70 eV.

Identification of the peaks was based on comparison of their mass spectra with the spectra of the WILEY library and in addition, in some cases, by comparison of their retention times with those of standard compounds. The Kovats indices were also calculated according to Tranchant (1982) and compared with available literature data (Kondjoyan and Berdagué, 1996). Peaks obtained are shown in Table 1. Semiquantitative determination of the volatile compounds was based on the ratio of their peak areas obtained from the total ion chromatogram, to that of dodecane (i.s.), and the results were expressed as ng dodecane /g dry matter.

Data analysis

Data analysis was carried out with SPSS program. Means of eigth determinations are shown (four distillations per brand of sausage and two injections per distillation were

carried out). ANOVA and a posteriory Tukey test were used to determine significant differences ($p<0.05$) among the five brands of sausages for every compound.

RESULTS AND DISCUSSION

The eficiency of the simultaneous steam ditillation-extraction method for its isolation of volatiles in meat products over dynamic headspace has been proven. Dirink et al. (1997) compared the two procedures for isolation of volatiles from hams concluding that SDE extraction have advantages especially due to the higher number of different volatiles isolated and better reproducibility.

One hundred ninety three different compounds were isolated from the five analyzed commercial brands of chorizo de Pamplona (Table 1). From a quantitative point of view, acids were the chemical family with the highest proportion in all brands, accounting for at least 60% of the total area. It was also the group that showed the lowest variability among the 5 brands (Coefficient of variation C.V.=10.95%). Long (C14-C18) and medium (C6-C12) chain fatty acids were found in all analyzed brands (except for hexanoic acid in brand 4). They come from the hydrolysis of triacylglycerols and phospholipids and from degradation of lipids, respectively (Girard and Bucharles,1991). These fatty acids can act as precursors of compounds affecting taste or aroma, but they are not directly responsible for sensory improvements in cured products (Arbolés and Juliá, 1992).

Short chain fatty acids ($C<6$) with greater implications in flavour development due to the very strong cheesy odours and to their lower threshold values have also been detected in some samples (brands 3 and 1). 2-methylpropanoic acid, 3-methylbutanoic acid and 2-methylbutanoic acid, which have been isolated from microbial metabolism of Val, Leu and Ile, respectively, have been attributed to a characteristic “sweet” odour (Mateo et al., 1996b). These 3 acids may also have a positive impact on aroma due to their conversion into fruity esters (Stahnke, 1994). Also butanoic acid, whose origin has

been poorly established was found in 4 of the 5 brands. It imparts a sour and cheesy note to the aroma (Stahnke, 1994). A similar percentage for the chemical group of acids (71%) for an industrial chorizo was found by Mateo et al. (1996b), but the profile of acids was different. Whereas oleic acid was the most abundant acid in 3 of the 5 brands analyzed in our work and palmitic in the 2 others, acetic acid was the most abundant one in their sausages. Acetic acid was not detected in this work, probably as a consequence of the employed method. Compounds like acetic acid, butanoic, 3-methylbutanoic and pentanoic acids could be present even if they have been not identified (Stahnke, 1994). In salami, some studies showed the presence of acetic acid (Berger et al., 1990; Schmidt and Berger, 1998), whereas Meynier et al. (1999) did not find it.

Butylated hydroxytoluene (E-321) and butylated hydroxyanisole (E-320) are additives of common use in food industry to prevent oxidation process. Butylated hydroxytoluene accounted for 0.03% to 14.04% of the total area in the five different brands. It was the third predominant compound in three brands. Its presence can be associated to both the addition of this substance to pig feed (Pascal and Desmoulin, 1973) and the direct addition (simultaneously to butylated hydroxyanisole) to the sausage mixture to act as an antioxidant.

Aldehydes accounted for percentages refered to the total area that ranged between 7.72% and 13.97%, which meant a C.V. among brands of 28.16%. Most of them come from lipid oxidation (hexanal, heptanal, 2-heptenal, octanal, 2-octenal, nonanal, 2-nonenal, 2,4-nonadienal, 2-decenal, 2,4-decadienal and 9-octadecenal) and certain off-flavours have been associated to them (Berdagué et al. 1993; Stahnke, 1994; Mac Leod, 1994). Also from lipid oxidation it has been detected 1-octen-3-ol, with a marked odour of mushroom and very low odour threshold, 2-heptanone (spicy, blue cheese odour) and n-alkanes with a poor contribution to aroma due to their high threshold values. As found

by Dirinck et al. (1997) in isolates of cured ham by SDE, we have also observed the presence of high molecular weight aldehydes (tetradecanal, pentadecanal, hexadecanal and octadecanal), but their importance to flavour development is only due to the fact that they can act as precursors of lower molecular weight alkanals and alkenals. Among other aldehydes, 2-phenylacetaldehyde (with a harsh, hawthorn odour) comes from the catabolism of phenylalanine (Berdagué et al., 1993) and it could serve as an indicator of proteolysis. Another compound from metabolism of amino acid was indol. Mateo et al. (1996b) associated this product to catabolism of tryptophan. A certain almond flavour has been associated with benzaldehyde, which was present in all sausages and is considered to be one of the substances that gives specific flavour notes in pork (Shahidi, 1994).

Terpenes were the chemical group that showed the highest differences among brands (range between 0.11% to 11.76% of the total area, with a C.V. among brands of 75.78%). 24 different terpenes have been isolated among the 5 analyzed brands, being only 17 identified and quantified. Although some of them (α-terpinene and limonene) have been found in meat as a consequence of their presence in animal feedstuffs, the major sources are related to the use of spices in preparation of sausages. Thus, molecules such as thujene, α-pinene, sabinene, α-phellandrene, 3-carene, γ-terpinene and α-terpinolene have been detected by Ekundayo et al. (1988) in pepper and β-caryophyllene, cubebene, 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). Other non terpenic compounds from spices found in sausages tested were eugenol (with spicy, honey-like odour), safrole and myristicine (spicy, nutmeg-odour) detected by Russel and Jennings (1969) in pepper and geranylacetone, β-ionone, methylsalicylate and tetramethylpyrazine detected in paprika by Guadayol et al. (1997).

Also due to the use of garlic as an ingredient, it has been possible to isolate sulphur compounds already detected in this spice by other authors (Kuo et al., 1990 and Mateo et al., 1996b) such as 1-propene,3,3'-thiobis, diallyl disulphide, diallyl trisulphide, 2-vinyl 4H-1,3-dithiin and methyl allyl disulphide. The variability observed among the brands in the content of terpenes showed that different amount of spices had been added in the production of the different brands.

One of the step involved in the elaboration process of industrial chorizo de Pamplona is smoking, which contributes to increase the phenolic fraction of the sausages due to the production of those compounds from pyrolysis of lignin (Hollenbeck, 1994). Also phenol is produced from benzaldehyde and phenylalanine by some bacteria (GenomeNet, 1998). Percentages of phenolic compounds for the samples ranged between 0.15% and 3.38% of the total area, with different profiles depending on the brand, and showing a C.V. among brands of 81.97%. As found by other authors, guaiacol and 4-methylphenol were 2 of the most predominant phenols detected, and have been associated with certain smoky and pungent flavour (Hollenbeck, 1994). Furthermore, due to their low odour thresholds, they would contribute considerably to the flavour of chorizo. Hydrocarbons such as toluene, xylene and ethylbenzene have also been previously detected in smoke aromas used to flavour processed meats (Wittkowski, 1989).

Numerous methyl and ethyl esters have been detected in one of the 5 brands, accounting for the 5% of the total area. They may have originated from esterification of alcohols and acids (Shahidi et al., 1986), with certain microbial involvement (Edwards et al., 1991; Stahnke, 1994).

In relation to the presence of alcohols, the most abundant one in 4 of the 5 brands was one with terpenic structure, the 4-terpineol. Linalool (floral odour), α-terpineol (peach

odour), geraniol (rose odour) and phenylethylalcohol (warm rose-honey odour) were other alcohols detected.

In summary, the study of the different volatile profiles showed evident flavour differences among commercial brands due to differences in the process conditions and in the type and amount of some ingredients. The greatest variability corresponded to esters (C.V. of 111%), related basically to the different starter cultures employed, phenols (C.V. of 82%) variability related specially to differences in the smoking process, and terpenes (C.V. of 76%), whose variability could be attributed to differences in the use of spices. The lowest variability corresponded to acids (C.V. of 11%) and aldehydes (C.V. of 28%).

RESULTS

Table 1. Profiles of volatile compounds of five brands of chorizo de Pamplona. (*)

KI	RI	Compound	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5
		Acids					
	C	Propanoic acid, 2-methyl			720		
	C	Butanoic acid	314b	75a	77a		115a
845	B	Butanoic acid, 3-methyl	75a		15211c		289b
881	B	Butanoic acid, 2-methyl			1870		
1001	A	Hexanoic acid	1851c	793a	963ab		1351b
1190	A	Octanoic acid	1661b	2206bc	3013c	352a	2962c
1386	A	Decanoic acid	2065a	9157b	12058bc	1413a	14336c
1573	A	Dodecanoic acid	1854b	1875b	2565bc	873a	3204c
1768	A	Tetradecanoic acid	3717a	11771c	7870b	3872a	10747bc
1949	A	9-Hexadecenoic acid	507a	6115c	2852b	2052ab	5860c
1975	A	Hexadecanoic acid	9747a	132108c	60336b	48407b	67081b
2157	A	9,12-Octadecadienoic acid	9492a	33031b	19423a	20437a	41002b
	A	9-Octadecenoic acid	26681a	70332bc	50837ab	65159b	99631c
		Subtotal	57966	267463	177797	142568	246580
			61,50%	*77,08%*	*68,56%*	*59,76%*	*61,71%*
		Alcohols					
833	C	1-Pentanol, 4-methyl			61		
853	C	2-Furanmethanol		235a	238a	191a	
868	B	Hexanol	59a	51a		70b	82c
881	C	Cyclohexanol	236				
984	B	1-Octen-3-ol	94a	272c	87a	89a	136b
1034	B	Hexanol, 2-ethyl	131c		87a	114b	
1038	C	Benzenmethanol	60a	105b			
1101	A	Linalool	362bc	198a	412c	312b	413c
1113	C	Phenylethylalcohol	118a	46a	1316b	206a	1074b
1120	B	2-Cyclohexen-1-ol, 1-methyl 4-(1-methylethyl)-*trans*					300
1165	B	Borneol	151ab		75a	150ab	234b
1176	C	4-Terpineol	2020a		2665a	2291a	3644b
1190	D	1,6-Octadien-3-ol / α-Terpineol	37a		216b	278b	
1262	B	Geraniol			82		
		Subtotal	3270	908	5241	3701	5885
			3,47%	*0,26%*	*2,02%*	*1,55%*	*1,47%*
		Aldehydes					
802	A	Hexanal	237b	357c	309bc	364c	145a
827	B	2-Furanocarboxyaldehyde	1168d	880c	218a	145a	472b
904	A	Heptanal	54a	83bc	98c	69ab	572d
959	B	Benzaldehyde	374d	131ab	167b	102a	214c
960	B	2-Heptenal		51a		64a	
1004	A	Octanal		153			
1012	B	2,4-Heptadienal				40	
1043	B	2-phenylacetaldehyde	1616c	1096b	933ab	633a	710a
1060	B	2-Octenal		78a		121b	
1105	B	Nonanal	490ab	513b	372a	442ab	538b
1161	B	(E)-2-Nonenal	63a	153b	142b	176b	198b
1213	B	2,4-Nonadienal				31	
1263	B	2-Decenal		96a	86a	114a	
1276	C	2-phenylacetaldehyde,α-ethylidene			91		
1318	B	2,4-Decadienal	24a		62ab	81bc	123c
1615	B	Tetradecanal	103a	134a	162a	136a	575b
1712	B	Pentadecanal	438a	880b	378a	438a	946b
1819	B	Hexadecanal	7055a	18615b	15328b	13116b	24981c
1999	C	9-Octadecenal	540a	1196bc	1425bc	1041b	1465c
2037	C	Octadecanal	1007a	2353c	2254bc	1678b	3194b
		Subtotal	13170	26771	22025	18792	34134
			13,97%	*7,72%*	*8,49%*	*7,88%*	*8,54%*

		Ketones					
892	B	2-Heptanone	74b	42a	64b	37a	
915	C	2(3H)-Furanone, dihydro		128			
956	C	2(3H)-Furanone, dihydro-5-methyl			92a	117a	202b
1118	C	2-Cyclohexen-1-one, 3,5,5-trimethyl					114
1140	B	Camphor	274b			93a	52a
1205	B	Verbenone	272a		277a	251a	
1458	B	Geranylacetone		51a		43a	483b
1489	B	b-Ionone	122a			232a	860b
1538	C	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4-4-7a-trimethyl		218a			505b
1661	C	2(3H)-Furanone, dihydro-5-(2-			291a	731b	
1682	C	g-dodecalactone	59a	290c		327c	218b
1696	B	2-Pentadecanone	263a	630bc	439ab	816c	1203d
1897	B	2-Heptadecanone	253a		223a	259a	204a
		Subtotal	1318	1360	1387	2906	3843
			1,40%	*0,39%*	*0,53%*	*1,22%*	*0,96%*
		Esters					
	C	Butanoic acid methyl ester			39		
804	B	Butanoic acid ethyl ester					88
810	C	Propanoic acid propyl ester					21
813	B	2-Hydroxypropanoic acid ethyl ester	91a				1207b
877	B	Acetic acid 3-methylbutyl ester					332
1003	B	Hexanoic acid ethyl ester	47a				41a
1062	C	2-Hydroxy-4-methylbutanoic acid ethyl ester					254
1127	A	Octanoic acid methyl ester	26a	37a	24a	74b	93c
1170	B	Benzoic acid ethyl ester					161
1191	C	Methyl salicylate	39a			51ab	67b
1199	B	Octanoic acid ethyl ester					690
1246	C	Benzeneacetic acid ethyl ester					86
1252	C	Hexanoic acid methylbutyl ester					98
1257	C	Acetic acid,2-pentyl ethyl ester					3770
1285	C	Bornyl acetate	53a		101ab	127b	134b
1298	C	Nonanoic acid ethyl ester					101
1329	A	Decanoic acid methyl ester	86a	175b	166b	155b	199b
1398	B	Decanoic acid ethyl ester	43a		25a	112a	3513b
1528	A	Dodecanoic acid methyl ester		161			
1597	B	Dodecanoic acid ethyl ester					930
1646	C	3-Methylbutyl decanoate					164
1723	A	Tetradecanoic acid methyl ester	83a	419d	79a	170b	277c
1795	B	Tetradecanoic acid ethyl ester	74a			23a	1952b
1928	A	Hexadecanoic acid methyl ester	1209c	1401c	338a	476ab	687b
1996	C	Hexadecanoic acid ethyl ester	54a	41a		59a	2598b
2111	A	9,12-Octadecadienoic acid methyl		180b	116a	189b	254c
2116	A	9-Octadecenoic acid methyl ester		33a	128c		69b
2171	C	9,12-Octadecadienoic acid ethyl ester					1463
	C	9-Octadecenoic acid ethyl ester					666
		Subtotal	1808	2447	1017	1437	19919
			1,92%	*0,71%*	*0,39%*	*0,60%*	*4,98%*
		Sulphur compounds					
848	B	1-Propene, 3,3'-thiobis	136a	234c	163ab	219bc	178abc
908	B	Propanal, 3-methylthio	189b	343c	206b	126a	219b
913	C	Methyl allyl disulphide	227b	208b	165a	356c	228b
1076	C	Diallyl disulphide	1074b	1930c	981ab	2304d	763a
1133	C	Sulphide allyl methyl	140b			100a	
1206	C	2-Vinyl-4H-1,3-dithiin					727
1296	C	Trisulphide, di-2-propenyl	322b	288b	151a	348b	675c
		Subtotal	2089	3004	1666	3454	2791
			2,22%	*0,87%*	*0,64%*	*1,45%*	*0,70%*
		Aromatic hydrocarbons					
	C	Toluene	75c	55ab	44a	46a	64bc
851	B	Ethylbenzene	11a	15a	25b	13a	
860	B	m-xylene	199ab	201ab	177ab	166a	233b
886	B	Styrene			26a	25a	
888	B	p-Xylene				35	

990	B	1,2,4-Trimethylbenzene	50b		21a		81c
1023	B	m-Cymene	265a		634b	514b	1342c
1176	C	Naphthalene	43				
1288	D	Methylnaphthalene		92b	19a	13a	8a
1304	D	Methylnaphthalene		68			
1530	D	Trimethylnaphthalene		62			
1566	D	Trimethylnaphthalene				18	
1592	D	Trimethylnaphthalene		62b		18a	
		Subtotal	644	557	945	849	1728
			0,68%	*0,16%*	*0,36%*	*0,36%*	*0,43%*
		Terpenes					
926	B	Thujene	266a		334ab	368b	385b
931	B	α-Pinene	1229bc	119a	1402c	1706d	1088b
945	B	Camphene	40a		45a	61ab	82b
972	A	Sabinene+β-Pinene	5109c	199a	7146d	10654c	2777b
992	B	β-Myrcene	311a		633c	747d	404b
1000	B	α-Phellandrene				185a	172a
1006	B	3-Carene	418a		2115c	1142b	383a
1014	B	α-Terpinene	87a			242b	681c
1026	A	Limonene	1951b	64a	2757d	2592d	2325c
1058	B	γ-Terpinene	604a		862b	968b	1338c
1086	B	α-Terpinolene	350a		511b	455b	493b
1210	D	Terpene			247		
1214	D	Terpene	97a				206b
1271	D	Terpene			nq		
1297	D	Terpene				nq	
1340	D	Terpene			75a	138b	nq
1352	B	Cubebene				53b	25a
1354	D	Terpene				nq	
1355	D	Terpene				nq	nq
1379	D	Copaene	43a		243c	611d	173b
1410	C	*Cis*-Caryophyllene	100a		367b		
1422	B	β-Caryophyllene	419a		1629c	2264d	846b
1440	C	α-Bergamotene			65a	85a	154b
1458	C	Humulene	60a		101b	140c	
		Subtotal	11087	382	18533	22413	11533
			11,76%	*0,11%*	*7,15%*	*9,40%*	*2,89%*
		Phenols					
995	B	Phenol	182a	703b	221a	291a	
1066	B	Phenol, 2-methyl	51a	643c	299b	253b	
1087	B	Phenol, 4-methyl	105a	2113c	428b	388b	
1089	B	Phenol, 2-methoxy (Guaiacol)	264a	1242c	544b	419b	
1109	B	Phenol, 2,5-dimethyl		46			
1148	B	Ethylphenol		66b	31a	23a	
1157	D	Phenol, 2,?-dimethyl	94a	486c	213b	242b	
1175	B	Ethylphenol		137			
1177	D	Phenol, 2,?-dimethyl		414			
1184	B	Ethylphenol		62			
1192	B	Phenol,4-methyl, 2-methoxy		510			
1229	C	4-Vinylphenol		258			
1234	D	Phenol ethyl-methyl		93			
1238	D	Phenol ethyl-methyl		68			
1245	D	Phenol, ethyl-methyl		104b		74a	
1248	B	Dimethoxyphenol		249b		67a	
1271	D	Phenol, trimethyl		56			
1276	D	Phenol, trimethyl		83			
1280	C	Phenol, 4-ethyl, 2-methoxy	58a	656c	321b	229b	
1316	B	Phenol, 4-Vinyl, 2-methoxy	64a	484b	157b	160b	100a
1359	C	Phenol 2,6-dimethoxy	97a	1050c	310b	415b	
1364	B	Eugenol	99a	155b	92a	139b	96a
1373	C	Phenol, 4-propyl, 2-methoxy		235			
1408	B	Methyleugenol	31a		219b	193b	195b
1412	C	Eugenol o isomer		42			
1444	D	Phenol,2,6-bis(1,1-dimethylethyl)			79a	104a	210b
1456	C	Eugenol o isomer	8a	160c	189d	67b	

1490	C	3-tert-butyl-4-methoxyphenol		1314			
1519	C	Phenol,2,4-bis(1,1-dimethylethyl)		152			
1609	C	Phenol, 2,6-dimethoxy-4-(2-		152b	69a	133b	
		Subtotal	1055	11737	3173	3198	602
			1,12%	*3,38%*	*1,22%*	*1,34%*	*0,15%*
		Nitrogen compounds					
1086	C	Tetramethylpyrazine		236b			173a
1294	B	Indole		38a	98a	47ab	67b
		Subtotal		273	98	47	240
				0,08%	*0,04%*	*0,02%*	*0,06%*
		Others					
806	B	Tetrachloroethylene	227b	100a	63a	90a	97a
992	B	2-Pentylfuran		176			
1120	D	2-Cyclohexen-1-ol,1-methyl 4-(1-methylethyl)-*trans*				183	
1151	C	Benzene,1,2-dimethoxy		230d	42b	90c	19a
1192	C	Benzene,1,4-dimethoxy				11	
1243	C	Dimethoxytoluene		116b		82a	
1287	B	Isosafrole	110b		15a	222c	215c
1317	C	Benzene,1,2,3-trimethoxy		532			
1408	C	Benzene,1,2,3-trimethoxy-5-methyl		357			
1478	C	2,6-di(t-butyl)-4-hydroxy -4-methyl-2,5-cyclohexadien-1-one			397a	173a	1884b
1483	C	2,6-di-t-butyl-4-methylene- 2,5-cyclohexadiene-1-one			190a	662a	2635b
1497	C	3-tert-Butyl-4-hydroxyanisole	13a	30042c	1972a	1787a	9019b
1515	C	Dibenzofurane		103			
1518	B	BHT	1120a	119a	22790b	32790c	56114d
1528	B	Myristicine	105a		724b	1006c	1736d
1562	C	Elemicine	104a		1209b	1279b	263a
1793	D	Anthracene				31	
		Subtotal	1679	31775	27403	38408	71982
			1,78%	*9,16%*	*10,57%*	*16,10%*	*18,01%*
		Alkanes					
800	B	Octane	20a	33a	39a	69b	
900	B	Nonane				nq	
1000	B	Decane	47a	64b		nq	
1100	B	Undecane				nq	
1200	B	Dodecane					
1300	B	Tridecane	30a			nq	116b
1400	B	Tetradecane	6a			160b	
1500	B	Pentadecane	70a			168b	
1600	B	Hexadecane				241a	238a
1700	B	Heptadecane		202b		145a	
1800	B	Octadecane					
		Subtotal	173	298	39	784	355
			0,18%	*0,09%*	*0,02%*	*0,33%*	*0,09%*
		Total	94259	346977	259325	238558	399592

(*) Results are mean values expressed in ng dodecane/g dry matter. In every brand, the percentage of each group refere to the total area of the compounds is shown.

KI: Kovats Indices for the DB5 column. **RI**: Reliability of identification, indicated by the following symbols:

A, mass spectrum and retention time identical with those of an autentic sample; B, mass spectrum and Kovats index in agreement with the corresponding literature data; C, mass spectrum consistent with spectra reported in the Wiley library data; D, tentative identification by mass spectrum. nq:not quantified.

Whithin a row, different letters denote significant differences ($p<0.05$) between the analyzed brands.

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