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Analytical Methods

Authentication study of volatile flavour compounds composition in Slavonian traditional dry fermented salami "kulen"

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ABSTRACT

Volatiles from famous Slavonian salami "kulen" were isolated by nitrogen purge and steam distillation (NPSD) and analysed, for the first time, by gas chromatography and mass spectrometry (GC and GC–MS). In all, 119 organic compounds were identified that originated from lipid oxidation, amino acid degradation, smoke treatment and added spices with different distribution among NPSD traps. NPSD method enabled comprehensive profiling with almost exclusive distribution of several major compounds in particular trap with little or no interference from abundant lipid constituents in the samples. The major identified flavour important compounds were methylphenols, methoxyphenols, organosulphur compounds (diallyl sulphide, diallyl disulphide, methylallyl disulphide, diallyl trisulphide and methional) and several derivatives of 2-cyclopenten-1-one such as ethyl cyclotene. Non-important flavour constituents such as high-molecular fatty acids, alcohols and aldehydes were also present among abundant compounds. General trends for possible discrimination of "kulen" volatiles profile among other European salami volatiles were noticed (lower amount of terpenes and higher percentages of diallyl sulphide, methoxyphenols, methylphenols and 2-cyclopenten-1-one derivatives).

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1. Introduction

Slavonian dry fermented salami "kulen" is a famous traditional meat product from Eastern Croatia without any additives such as nitrites or ascorbic acid and with excellent international consumer acceptance. It is produced according to traditional procedure from minced pork, with the addition of salt, red paprika, hot paprika and garlic. Specific olfactory and gustatory properties of this salami have the highest influence on the consumer perception of quality. Similar products from Spain (Chorizo de Pamplona and Salchichón) and Italy (Felino and Milano salami) have been intensively studied for their physical–chemical composition and olfactory and gustatory properties (Dellaglio, Casiraghi, & Pompei, 1996; Gimeno, Ansorena, Astiasarán, & Bello, 2000; Perez-Alvarez, Sayes-Barbare, Fernandez-Lopez, & Aranda-Catala, 1999).

During the processing of dry-cured meat products numerous enzymatic and nonenzymatic reactions occur such as protein degradation, lipid degradation and oxidation, Maillard reactions and Strecker degradation. These changes give rise to volatile compounds such as aldehydes, carboxylic acids, alcohols, ketones, esters, as well as sulphur, nitrogen and other compounds (Mottram, 1998; Toldrá, 1998; Vestergaard, Schivazappa, & Virgili, 2000). Different techniques have been applied for the extraction of these compounds from dry fermented meat products such as steam distillation (SD), simultaneous distillation extraction (SDE), solvent extraction (SE), dynamic headspace (DHS), purge and trap techniques and more recently headspace solid-phase microextraction (HS-SPME) (Ai-Nong & Bao-Guo, 2005; Carrapiso, Ventanas, & García, 2002; Wilkes et al., 2000; Yu, Sun, Tian, & Qu, 2008). It was observed that a fundamental difference between fermented sausages and dry-cured ham is that sausage processing allows the distribution of ingredients inside the product, not only on the surface. Consequently, the microflora of fermented sausages has much better opportunities to influence the flavour formation. Approximately 400 volatiles have been identified in fermented sausage (Stahnke, 2002), from dry-cured ham the number is approximately 200 (Ruiz, Muriel, & Ventanas, 2002). However, many of those volatiles do not contribute to aroma because of high sensory threshold values. The composition and quantity of these volatiles are affected by the meat type, added spices, smoke treatment, length of the curing process and others resulting in a variety of flavour tones (Andrés, Cava, & Ruiz, 2002; Ruiz, Ventanas, Cava, Andrés, & Garciá, 1999). In spite of to the negative effect of lipid oxidation, the

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typical aroma of dry-cured meat products is related to the initiation of lipid oxidation and the subsequent generation of volatiles (Buscailhon, Berdagué, & Monin, 1993). Smoke-cured products also contain low-molecular weight phenol derivatives (lignin monomer derivatives) originated from the smoke or smoke flavourings (Ai-Nong & Bao-Guo, 2005; Guillén & Ibargoitia, 1999).

On the basis of these remarks, this study was firstly aimed at the characterisation of "kulen" volatiles (no previous papers were found) with the objective of authenticating this traditional famous salami and determination of its volatile chemical fingerprint. For this purpose, nitrogen purge and trap distillation (NPSD) was used followed by GC and GC–MS analyses as in previous paper (Jerković, Mastelić, & Tartaglia, 2007). Isolated volatiles were compared with the published papers on other typical European salami volatiles indicating general trends for possible "kulen" discrimination taking into consideration strong impact of different isolation methods to the obtained qualitative and quantitative volatiles composition. These results may be helpful as a tool for the certification of this autochthonous Croatian food product as one step toward complying EU law concerning Protection of Denomination of Origin (DOP) and of Geographical Origin (IGP).

2. Materials and methods

2.1. Materials and reagents

Six samples of traditional dry fermented salami "kulen" were collected from different producers in Eastern Croatia (Slavonia). All samples had been prepared according to the traditional processing procedure without any additives such as nitrites or ascorbic acid. The procedure starts in November and last until June or July. "Kulen" is made from pig meat at least 12 months old, only from the highest quality parts of the pig, such as the thigh, the back and *c*. 20% of shoulder. The meat is cut into stripes 30 cm long, 10 cm wide and 3 cm thick and placed in a freezer until the temperature of the meat reaches −2 to −5 °C. The meat is than grinded through a grinding plate with holes of 8–12 mm in diameter. The grinded meat is then mixed with salt in the amount of 2%, red paprika in the amount of 1%, hot paprika in the amount of 0.7% and garlic in the amount of 0.2%. The minced meat is then stuffed into a pig appendix and tied with hemp rope. Thereafter, "kulen" is smoked with dry hard wood (hornbeam and beech) every second day for two weeks. The temperature and relative humidity at this stage should be 18–20 °C and 70–90%. After the smoking, "kulen" is left for ripening more than 6 months in a dark room with the temperature from 14 to 17 °C and relative humidity 70–80%.

All of the solvents employed (p.a. grade), anhydrous sodium sulphate and series of alkanes, alcohols, acids and phenols were purchased from Fluka Chemie (Buchs, Switzerland). Ether was dried over K_2CO_3 and distilled. Oxygen free nitrogen gas was purchased from Messer, Dugi Rat, Croatia.

2.2. Nitrogen purge and steam distillation (NPSD)

The apparatus for NPSD comprised of a 100 mL two-neck flask for the inlet of the purging nitrogen gas and for outlet of the nitrogen containing the purged volatiles, the later was connected to the cold traps (Ai-Nong & Bao-Guo, 2005; Ramarathnam, Rubin, & Diosady, 1993). The sample (65 g) cut in small thin pieces was placed in the flask where it was constantly maintained at 102 ± 5 °C with the use of an oil bath. A slow stream of nitrogen was passed through the sample to purge the volatiles from the headspace as in previous paper (Jerković et al., 2007). The effluent stream was condensed through a series of cold traps. The first cold trap (1st trap) was maintained at a temperature of 2–4 °C with crushed ice; the second cold trap (2nd trap), containing 30 mL of ether, was maintained at −20 °C with temperature cooling bath Huber with thermoregulator CC1 (Offenburg, Germany); and the third cold trap (3rd trap), containing 30 mL of *n*-pentane, was also maintained at −20 °C in the bath. The volatiles were collected over a 10 h purging and distilling period. At the end of the experiment, the volume of the condensate collected at the first cold trap was *c.* 30 mL. This condensate was extracted three times with 30 mL diethyl ether. The ether extracts were combined, dried over anhydrous sodium sulphate and concentrated by fractional distillation to a final volume of 0.2 mL. The extracts from the second and the third cold trap were also dried and concentrated by fractional distillation up to 0.2 mL. All the concentrates were analysed by GC and GC–MS.

2.3. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analyses

GC analyses were performed on an Agilent Technologies (Paolo Alto, CA, USA) gas chromatograph model 7890A. GC–MS analyses were performed on an Agilent Technologies (Paolo Alto, CA, USA) gas chromatograph model 7890A with a mass selective detector model 5975A. GC operating conditions were: column HP-5MS (Agilent J&W GC column), 30 m × 0.25 mm i.d., film thickness 0.25 μm; column temperature programmed from 70 °C isothermal for 2 min, then increased to 200 °C at a rate of 3 °C min^{-1} and held isothermal for 15 min. Carrier gas was helium at flow rate 1 mL min^{-1}. Injector temperature was 250 °C. Volume injected was 1 μL and split ratio was 1:50. MS conditions were: ionisation voltage 70 eV; ion source temperature 280 °C; mass scan range: 30–300 mass units.

2.4. Identification and quantitative determination of components

The individual peaks were identified by comparison of their retention indices (relative to C_9–C_{25} *n*-alkanes) to those of standards (series of alkanes, alcohols, acids and several phenols) and literature data (El-Sayed, 2007 and references therein; Adams, 1995), as well as by comparing their mass spectra with the Wiley 6.0 library (Wiley, New York) and NIST98 (National Institute of Standards and Technology, Gaithersburg) mass spectral database. Determination of the percentage composition was based on peak area normalisation (expressing the area of a given peak as a percentage of the sum of the areas of all the peaks) without the use of correction factors. The component percentages in Table 1 and standard deviations were calculated from duplicate GC and GC–MS analyses of six "kulen" samples.

3. Results and discussion

Nitrogen purge and steam distillation (NPSD) was used to isolate the headspace volatiles of Slavonian dry fermented salami "kulen" into three different fractions depending on their volatility and solubility. The presence of interfering compounds for the identification of flavour important compounds, a peculiar case is large amounts of fat (lipid matrix), is a problem and required the use of suitable isolation procedure. Namely, high molecular fat derived compounds present in the samples with high concentration, have a high perception threshold and play a small role in total perceived flavour in distinction from the lower-molecular weight compounds present in small concentration. Applied NPSD method exhibited little or no interference from medium volatile lipid constituents (with low headspace concentration). Volatile compounds from lipid oxidation, amino acid degradation, smoke treatment and added spices have been isolated and identified with different distribution in the traps that enabled comprehensive volatiles profiling.

Table 1
Volatile compounds isolated by nitrogen purge and steam distillation (NPSD).

No.	Compounds		1st Trap Peak area (%)				2nd Trap Peak area (%)				3rd Trap Peak area (%)			
		RI_1	Min.	Max.	Av.	SD	Min.	Max.	Av.	SD	Min.	Max.	Av.	SD
Organosulphur compounds														
1.	Diallyl sulphide	<900	–	–	–	–	–	–	–	–	0.0	1.6	0.85	0.67
2.	Methional	908	–	–	–	–	0.0	0.4	0.18	0.21	–	–	–	–
3.	Methyl allyl disulphide	921	–	–	–	–	0.6	2.1	1.28	0.64	–	–	–	–
4.	Diallyl disulphide	1082	–	–	–	–	20.6	31.2	26.75	4.88	0.0	6.5	2.88	2.72
5.	Diallyl trisulphide	1298	–	–	–	–	0.0	0.4	0.25	0.17	–	–	–	–
Carbonyl compounds														
6.	2-Heptanone	<900	–	–	–	–	0.0	0.2	0.08	0.09	–	–	–	–
7.	Heptanal	903	–	–	–	–	0.0	0.8	0.33	0.39	0.0	0.2	0.08	0.10
8.	2-Methyl-2-cyclopenten-1-one	909	0.0	0.1	0.05	0.06	0.0	0.5	0.28	0.22	–	–	–	–
9.	Benzaldehyde	964	0.0	0.1	0.05	0.06	0.5	0.6	0.50	0.08	–	–	–	–
10.	3-Methyl-2-cyclopenten-1-one	967	0.3	0.4	0.35	0.06	0.0	0.5	0.20	0.25	–	–	–	–
11.	Octanal	1004	–	–	–	–	0.3	0.8	0.50	0.22	0.2	0.2	0.18	0.05
12.	Nonanal	1106	0.4	0.5	0.43	0.05	0.5	2.3	1.15	0.79	0.8	1.1	0.98	0.13
13.	2-Hydroxy-3-methyl-2-cyclopenten-1-one	1032	0.0	1.6	0.83	0.66	0.0	0.6	0.35	0.26	–	–	–	–
14.	2,3-Dimethyl-2-cyclopenten-1-one	1042	0.3	0.6	0.45	0.13	0.0	0.4	0.18	0.21	–	–	–	–
15.	Phenylacetaldehyde	1047	0.2	0.4	0.30	0.08	0.2	1.3	0.63	0.47	0.0	0.3	0.13	0.15
16.	3,5-Dimethyl-cyclopentenolone	1061	0.0	0.4	0.18	0.21	–	–	–	–	–	–	–	–
17.	3-Ethyl-2-cyclopenten-1-one	1083	0.0	0.2	0.08	0.09	–	–	–	–	–	–	–	–
18.	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	1123	0.0	0.6	0.23	0.29	–	–	–	–	–	–	–	–
19.	Decanal	1207	0.1	0.2	0.13	0.05	0.0	0.2	0.10	0.08	0.2	0.5	0.33	0.13
20.	2-Hydroxy-3-propyl-2-cyclopenten-1-one	1211	0.0	0.1	0.08	0.10	–	–	–	–	–	–	–	–
21.	4-Hydroxy-3-methoxy-benzaldehyde	1398	0.0	0.2	0.10	0.08	–	–	–	–	–	–	–	–
22.	2,6-di(*tert*-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	1475	0.0	0.2	0.13	0.09	0.0	0.9	0.55	0.40	–	–	–	–
23.	Hexadecanal	1820	5.0	5.5	5.08	0.30	0.0	1.9	0.88	0.78	0.0	0.5	0.33	0.22
24.	(*Z*)-9-Octadecenal	1995	0.0	0.6	0.30	0.25	–	–	–	–	–	–	–	–
Alcohols														
25.	2-Buthoxyethanol	907	0.0	0.1	0.05	0.06	–	–	–	–	–	–	–	–
26.	1-(2-Methoxypropoxy)-2-propanol	1017	0.0	0.4	0.23	0.17	–	–	–	–	–	–	–	–
27.	Benzyl alcohol	1039	0.0	0.2	0.08	0.09	–	–	–	–	–	–	–	–
28.	2-Phenylethanol	1117	0.0	0.9	0.53	0.39	–	–	–	–	–	–	–	–
29.	3-Isopropyl-4-methyl-1-decen-4-ol[a]	1301	0.0	0.2	0.10	0.08	–	–	–	–	–	–	–	–
30.	1-Hexadecanol	1882	1.4	7.5	5.18	2.73	1.2	7.9	5.23	2.94	10.2	21.2	17.35	4.95
31.	(*Z*)-9-Octadecen-1-ol	2069	2.4	20.1	11.88	7.54	15.8	20.5	18.33	1.97	23.8	32.4	27.80	4.08
32.	1-Octadecanol	2087	0.1	0.5	0.35	0.17	0.0	3.2	1.73	1.49	1.1	8.8	4.98	3.14
Terpenes														
33.	α-Pinene	934	0.1	0.1	0.10	0.01	0.4	0.5	0.43	0.05	0.0	0.6	0.33	0.25
34.	Δ-3-Carene	1014	–	–	–	–	0.0	0.3	0.15	0.13	–	–	–	–
35.	Limonene	1033	–	–	–	–	0.4	1.0	0.75	0.25	0.0	0.4	0.18	0.21
36.	β-Ionone	1487	0.0	0.2	0.12	0.08	–	–	–	–	–	–	–	–
Aliphatic hydrocarbons														
37.	Decane	1000	–	–	–	–	–	–	–	–	0.0	0.6	0.28	0.32
38.	(E,*Z*)-3,4-Dimethyl-2,4-hexadiene	1025	0.0	0.1	0.05	0.06	–	–	–	–	–	–	–	–
39.	2,3,7-Trimethyldecane	1060	–	–	–	–	–	–	–	–	0.0	2.4	1.05	0.99
40.	Undecane	1100	0.0	0.6	0.23	0.29	0.0	1.6	0.85	0.67	0.0	0.3	0.20	0.14
41.	4-Methylundecane[a]	1103	–	–	–	–	–	–	–	–	0.8	1.1	0.95	0.13
42.	Naphthalene	1184	–	–	–	–	0.2	0.9	0.48	0.31	0.0	0.4	0.25	0.17
43.	Dodecane	1200	–	–	–	–	0.0	0.3	0.13	0.15	–	–	–	–
44.	Tridecane	1300	0.0	1.3	0.70	0.53	1.9	2.4	2.08	0.22	0.0	1.2	0.63	0.51
45.	Tetradecane	1400	0.0	0.2	0.10	0.08	–	–	–	–	–	–	–	–
46.	Pentadecane	1500	0.4	0.7	0.63	0.15	1.0	1.1	1.03	0.05	0.4	0.5	0.43	0.05

(*continued on next page*)

Table 1 (*continued*)

No.	Compounds		1st Trap Peak area (%)				2nd Trap Peak area (%)				3rd Trap Peak area (%)			
		RI_1	Min.	Max.	Av.	SD	Min.	Max.	Av.	SD	Min.	Max.	Av.	SD
47.	Hexadecane	1600	0.0	0.1	0.08	0.05	0.0	0.5	0.30	0.22	0.0	0.2	0.10	0.08
48.	Heptadecane	1700	0.0	0.2	0.10	0.08	–	–	–	–	–	–	–	–
49.	Heneicosane	2100	0.0	0.1	0.08	0.05	–	–	–	–	0.0	0.8	0.45	0.33
Aromatic hydrocarbons														
50.	Ethylbenzene	<900	–	–	–	–	–	–	–	–	0.0	0.5	0.30	0.22
51.	1,3-Dimethylbenzene	<900	–	–	–	–	–	–	–	–	0.0	2.2	1.00	0.90
52.	Ethenylbenzene	<900	–	–	–	–	0.0	0.3	0.13	0.15	0.0	0.1	0.05	0.06
53.	1,2-Dimethylbenzene	<900	–	–	–	–	–	–	–	–	0.0	0.6	0.33	0.25
54.	2-Phenyl-1-propene	985	–	–	–	–	0.0	0.1	0.05	0.06	–	–	–	–
55.	1,3,5-Trimethylbenzene	997	–	–	–	–	0.0	0.5	0.20	0.25	–	–	–	–
56.	1,4-Dimethoxybenzene	1187	0.0	0.3	0.20	0.14	–	–	–	–	0.0	0.3	0.18	0.13
57.	1,4-bis(1,1-Dimethylethyl)-benzene	1395	–	–	–	–	–	–	–	–	0.0	0.3	0.18	0.13
58.	1,2,3-Trimethoxy-5-methylbenzene	1407	0.1	0.4	0.28	0.13	–	–	–	–	–	–	–	–
59.	*p*-di-*tert*-Pentylbenzene	1490	–	–	–	–	–	–	–	–	0.3	0.5	0.35	0.10
60.	2,3,5-Trimethoxytoluene	1529	0.0	0.5	0.30	0.22	–	–	–	–	–	–	–	–
Carboxylic acids														
61.	Pentanoic acid	<900	0.0	0.1	0.05	0.06	–	–	–	–	–	–	–	–
62.	Hexanoic acid	973	0.3	0.6	0.38	0.17	–	–	–	–	–	–	–	–
63.	2-Ethylhexanoic acid	1120	0.8	1.2	1.00	0.16	0.3	0.5	0.40	0.08	1.4	5.0	3.05	1.48
64.	Octanoic acid	1183	0.4	1.2	0.75	0.34	–	–	–	–	–	–	–	–
65.	Nonanoic acid	1285	0.0	0.2	0.15	0.10	–	–	–	–	–	–	–	–
66.	Decanoic acid	1379	0.0	4.3	1.78	1.87	–	–	–	–	–	–	–	–
67.	Dodecanoic acid	1574	0.0	1.1	0.75	0.51	–	–	–	–	–	–	–	–
68.	Tetradecanoic acid	1776	0.8	3.6	2.28	1.18	–	–	–	–	0.0	0.4	0.25	0.17
69.	Pentadecanoic acid	1867	0.0	0.2	0.13	0.10	–	–	–	–	–	–	–	–
70.	Hexadecanoic acid	1980	1.0	8.4	4.28	3.14	0.0	2.1	1.03	0.86	1.2	5.9	3.78	1.94
71.	(*Z*)-9-Octadecenoic acid	2167	1.0	7.8	4.95	2.88	0.0	0.5	0.38	0.25	–	–	–	–
Phenols														
72.	Phenol	980	2.7	4.9	3.53	0.97	0.6	1.6	1.10	0.42	0.0	0.4	0.18	0.21
73.	2-Methylphenol	1056	1.5	1.8	1.65	0.13	0.2	1.2	0.68	0.43	0.0	0.4	0.18	0.21
74.	3-Methylphenol	1077	1.5	4.4	2.78	1.29	0.0	1.4	0.65	0.58	0.0	0.4	0.18	0.21
75.	2-Methoxyphenol	1093	2.1	11.2	7.28	3.79	2.0	2.1	2.03	0.05	0.0	0.6	0.30	0.25
76.	2,6-Dimethylphenol	1109	0.0	0.2	0.10	0.08	–	–	–	–	–	–	–	–
77.	2-Ethylphenol	1142	0.0	0.3	0.13	0.15	–	–	–	–	–	–	–	–
78.	2,4-Dimethylphenol[b]	1151	0.6	1.0	0.80	0.16	0.0	1.0	0.53	0.41	–	–	–	–
79.	2,5-Dimethylphenol[b]	1154	0.7	0.9	0.80	0.08	–	–	–	–	–	–	–	–
80.	4-Ethylphenol	1170	0.0	0.5	0.30	0.22	–	–	–	–	–	–	–	–
81.	3-Ethylphenol	1173	0.0	1.2	0.68	0.50	–	–	–	–	–	–	–	–
82.	2,3-Dimethylphenol[b]	1181	0.0	0.7	0.40	0.32	0.4	0.5	0.43	0.05	0.0	0.9	0.48	0.37
83.	2-Methoxy-4-methylphenol	1195	3.4	7.7	4.83	1.95	0.3	1.2	0.78	0.37	–	–	–	–
84.	2,4,6-Trimethylphenol[b]	1205	0.0	0.2	0.10	0.08	–	–	–	–	–	–	–	–
85.	2-Ethyl-6-methylphenol	1232	0.0	0.2	0.15	0.10	–	–	–	–	–	–	–	–
86.	2-Ethyl-5-methylphenol	1242	0.0	0.8	0.43	0.30	–	–	–	–	–	–	–	–
87.	3-Ethyl-5-methylphenol	1247	0.0	0.3	0.15	0.13	–	–	–	–	–	–	–	–
88.	2,3,5-Trimethylphenol[b]	1270	0.0	0.5	0.28	0.21	–	–	–	–	–	–	–	–
89.	4-Ethyl-2-methoxyphenol	1282	2.9	3.1	3.00	0.08	0.0	0.7	0.38	0.29	–	–	–	–
90.	4-Vinyl-2-methoxyphenol	1315	0.0	1.2	0.55	0.49	–	–	–	–	–	–	–	–
91.	2,6-Dimethoxyphenol	1357	0.3	4.1	2.05	1.56	0.0	0.5	0.30	0.22	–	–	–	–
92.	2-Methoxy-4-(2-propenyl)-phenol	1361	0.5	0.6	0.53	0.05	–	–	–	–	–	–	–	–
93.	2-Methoxy-4-propylphenol	1370	0.0	0.4	0.23	0.17	–	–	–	–	–	–	–	–
94.	4-Methyl-2,6-bis(1,1-dimethylethyl)-phenol	1515	3.4	4.5	3.48	0.71	6.0	7.1	5.78	1.00	0.4	0.6	0.45	0.10
Esters and lactones														

95.	γ-Butyrolactone	915	–	–	–	–	0.0	1.2	0.63	0.51	–	–	–	–
96.	Dihydro-5-methyl-2-(3H)-furanone (γ-valerolactone)	956	–	–	–	–	0.0	0.4	0.15	0.19	–	–	–	–
97.	Pantolactone	1040	0.0	0.2	0.08	0.09	–	–	–	–	–	–	–	–
98.	2,3-Dimethyl-4-hydroxy-2-butenoic lactone	1113	0.0	0.1	0.05	0.06	–	–	–	–	–	–	–	–
99.	Butoxyethoxyethyl acetate	1372	0.3	1.2	0.75	0.42	1.3	1.8	1.43	0.35	0.6	1.1	0.93	0.22
100.	Methyl tetradecanoate	1727	0.0	0.1	0.05	0.06	–	–	–	–	–	–	–	–
101.	Isobuthyl phthalate	1869	0.0	0.2	0.13	0.09	–	–	–	–	0.5	1.3	0.90	0.34
102.	Methyl hexadecanoate	1928	0.0	0.1	0.06	0.05	–	–	–	–	–	–	–	–
103.	Dibutyl phthalate	1963	0.3	1.8	0.98	0.62	0.0	1.2	0.75	0.53	–	–	–	–
104.	Dioctyl adipate	>2500	0.0	0.2	0.15	0.10	–	–	–	–	–	–	–	–
Furans, pyrans and benzofurans														
105.	2-Furanmethanol	<900	0.0	1.9	0.70	0.84	–	–	–	–	0.0	0.5	0.25	0.21
106.	2-Pentylfuran	993	–	–	–	–	0.5	1.1	0.85	0.26	0.0	0.6	0.23	0.29
107.	2-Furfuryl acetate	995	–	–	–	–	0.0	0.4	0.15	0.19	–	–	–	–
108.	Benzofuran	1001	–	–	–	–	0.0	0.5	0.20	0.25	–	–	–	–
109.	5,6-Dihydro-4-methyl-2H-pyran-2-one	1165	0.0	0.2	0.10	0.08	–	–	–	–	–	–	–	–
110.	2,3-Dihydrobenzofuran	1223	0.0	0.5	0.25	0.21	–	–	–	–	–	–	–	–
111.	Dibenzofuran	1509	0.0	0.1	0.05	0.06	–	–	–	–	–	–	–	–
Organonitrogen compounds														
112.	Benzonitrile	988	–	–	–	–	0.0	0.3	0.15	0.17	–	–	–	–
113.	2-Ethyl-6-methyl-pyrazine	1001	0.0	0.1	0.05	0.06	–	–	–	–	–	–	–	–
114.	2-Acetylpyrrole	1067	0.0	1.3	0.53	0.64	0.1	0.4	0.28	0.13	–	–	–	–
115.	2,3-Dihydro-1H-inden-1-ol	1234	0.0	0.4	0.23	0.17	–	–	–	–	–	–	–	–
116.	2,3-Dihydro-3-methyl-1H-inden-1-one	1305	0.0	0.1	0.05	0.06	–	–	–	–	–	–	–	–
117.	N,N-Dibutyl-acetamide	1359	–	–	–	–	0.3	0.9	0.60	0.26	0.0	0.5	0.28	0.21
118.	Hexadecanamide	2207	0.0	0.5	0.30	0.22	–	–	–	–	–	–	–	–
119.	Octadecanamide	>2500	0.0	0.4	0.25	0.17	–	–	–	–	–	–	–	–
Total identified (%)			88.5–92.9%				86.3–93.3%				85.3–95.5%			

Min. – minimal percentage, Max. – maximal percentage, Av. – average percentage; SD – standard deviation, RI – retention indices on HP-5MS column, (–) not detected.

[a] Tentatively identified.

[b] Correct isomer not identified.

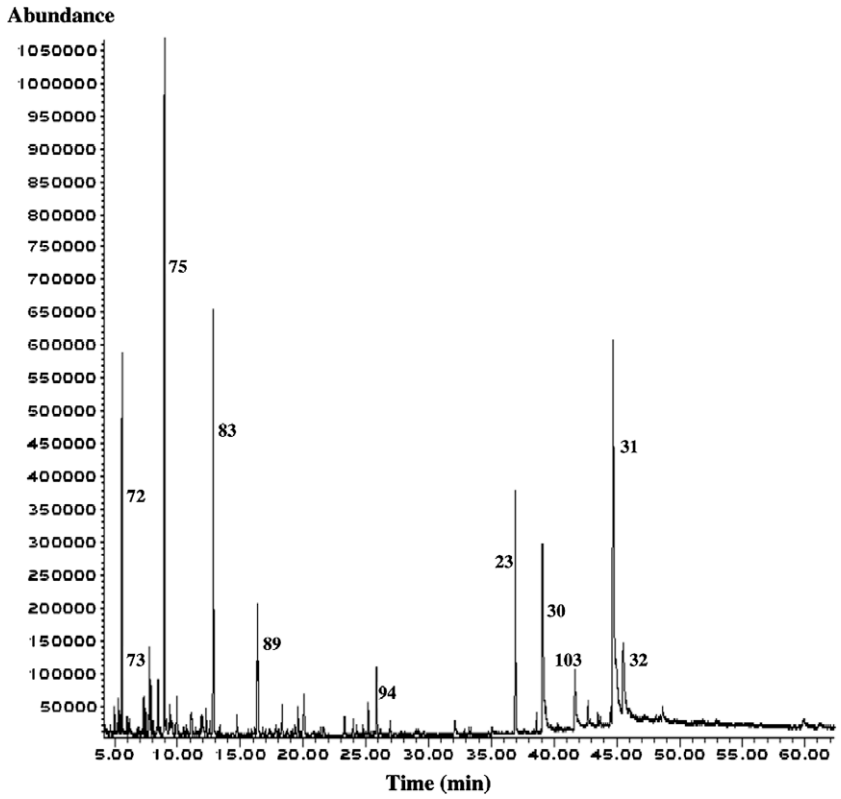

Fig. 1. Representative total ion chromatogram (TIC) of the diethyl ether extract from the 1st trap on HP-5MS column. Numbers refer to major compounds in Table 1.

Distribution among several isolated flavour impact compounds was almost exclusive in the particular trap (Figs. 1, 2 and 4; Table 1). Although NPSD was done at 102 ± 5 °C the formation of thermal oxidation artefacts is not promoted, since nitrogen not only serves

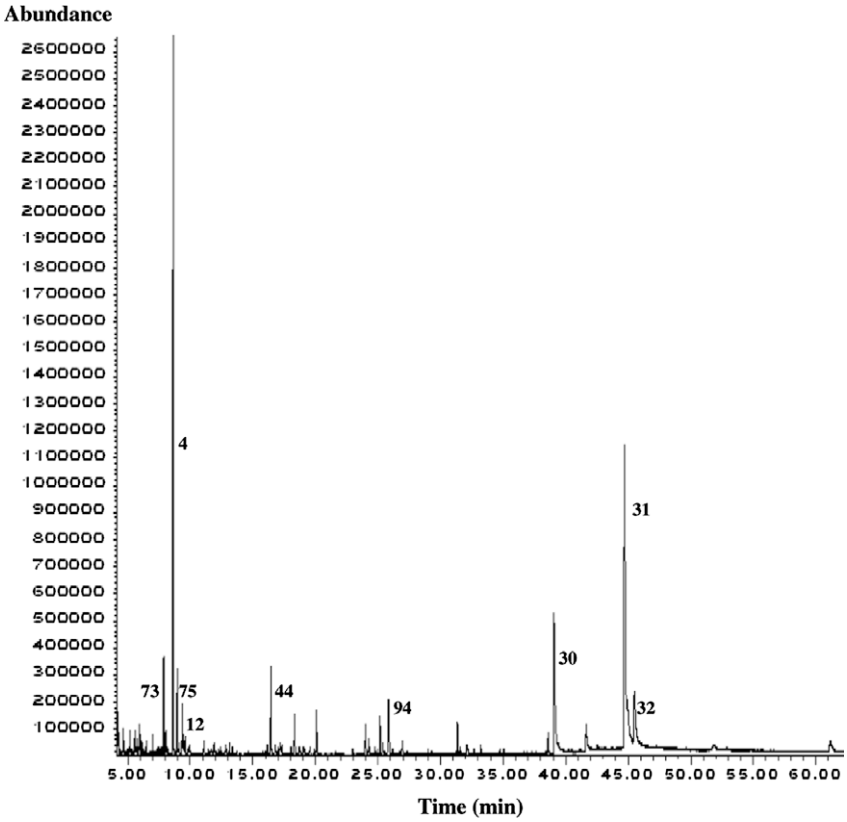

Fig. 2. Representative total ion chromatogram (TIC) of the diethyl ether extract from the 2nd trap on HP-5MS column. Numbers refer to major compounds in Table 1.

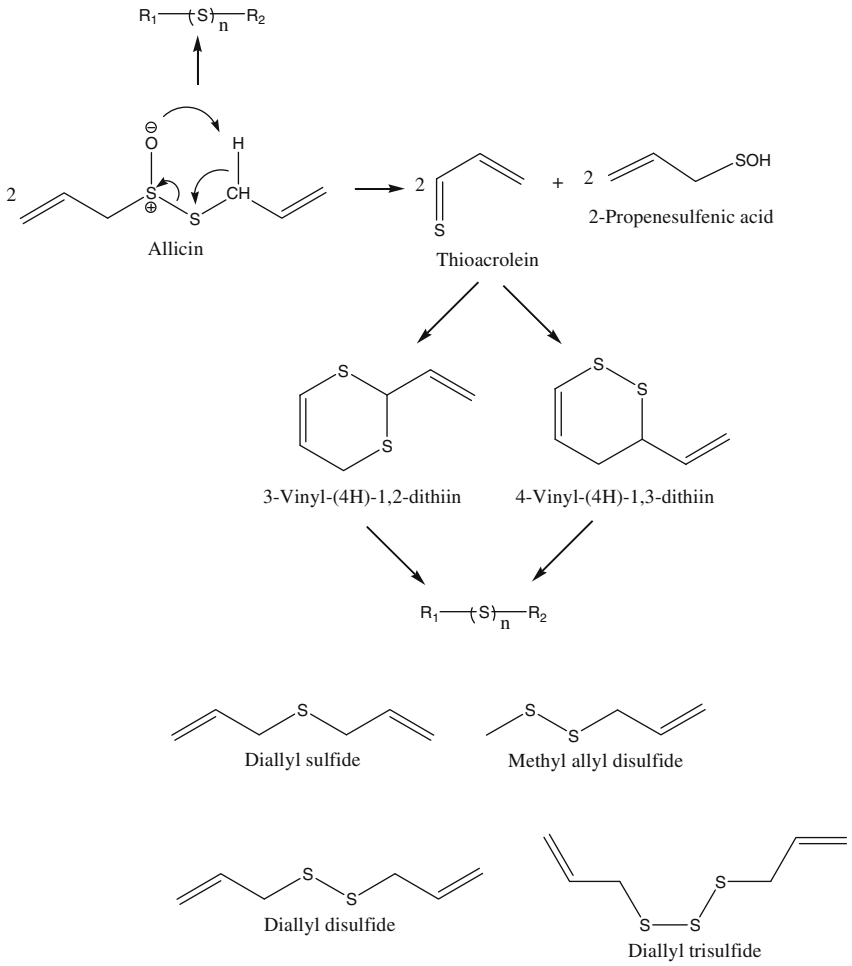

Fig. 3. Allicin decomposition and structures of identified sulphides.

as a carrier gas, but also provides an inert medium which prevents oxidation of unsaturated fatty acids, sugars and amino acids (Ai-Nong & Bao-Guo, 2005; Ramarathnam et al., 1993).

As it is known, flavour compounds developed from the autooxidation of lipids give to food globally rancid or oxidised descriptors, depending on the reached oxidation level. However, they were not identified with high percentages in "kulen" samples that can be linked to smoking process with one of the aims to prevent the lipid oxidation by adding to the salami some antioxidants presents in wood smoke as phenolic compounds. As was expected from a smoked meat product compounds with different functional groups were isolated including phenolic compounds, furans and 2-cyclopenten-1-one derivatives, all of which are characteristic of wood smoke derived from cellulose, hemicellulose and lignin pyrolysis (Maga, 1987; Toth & Potthast, 1984). In the smoking process the smoke components are adsorbed by kulen surface, and they react or establish more or less strongly interactions with salami components. As the consequence of this, the headspace of the smoked salami only will contain those smoke components, adsorbed on the surface, which have not reacted or interacted very strongly. It was already reported that carbohydrate derivatives (levoglucosane and others of similar nature) and pyrocatechol derivatives are generally present in smoke but they were not detected in the headspace of smoked food products and carbonyl, carboxyl and phenol derivatives were present but in proportions very different to those usually found in smoke (Guillén, Errecalde, Salmerón, & Casas, 2006).

The first cold trap mainly served to condense the water vapour and collect the condensed components. Representative total ion chromatogram of extracted volatiles from first trap is presented in Fig. 1. Eighty-nine compounds were identified (Table 1) including mostly fatty acids and alcohols derived from lipid oxidation and phenols from smoke. Among identified higher fatty acids, alcohols and aldehydes, the components with the highest area percentage were (*Z*)-9-octadecen-1-ol (2.4–20.1%), hexadecanoic acid (1.0–8.4%), (*Z*)-9-octadecenoic acid (1.0–7.8%), 1-hexadecanol (1.4–7.5%), hexadecanal (5.0–5.5%), decanoic acid (0.0–4.3%) and tetradecanoic acid (0.8–3.6%). Those compounds were expected to be identified since lipids show intensive lipolysis during dry-curing, especially during salting and post-salting, whilst the free fatty acids accumulate as a result of triglycerides hydrolysis (Moltiva, Toldrà, Nieto, & Flores, 1993). Due to their low volatility, their contribution to salami flavour is of less importance (Dirinck, Van Opstaele, & Vandendriessche, 1997). In addition, lower aliphatic fatty acids were also detected such as pentanoic acid (0.0–0.1%), hexanoic acid (0.3–0.6%), octanoic acid (0.4–1.2%), nonanoic acid (0.0–0.2%), decanoic acid (0.0–4.3%) and dodecanoic acid (0.0–1.1%). The C2–C6 acids can also be derived from microbial degradation of pyruvate (Gottschalk, 1986). Lower lipid oxidation alcohols were not identified, whilst known breakdown products of hydroperoxides nonanal as well as decanal were present with low percentages. Smoke derived phenols were the most abundant class (with respect to the overall number) of volatile organic compounds in the first trap. Besides phenol (2.7–4.9%), the major identified

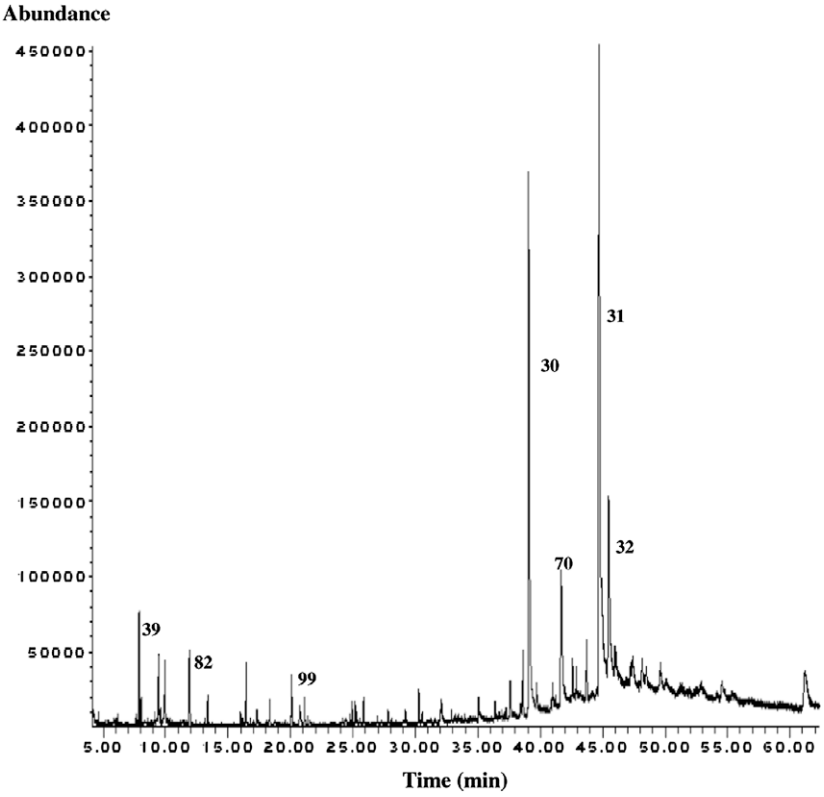

Fig. 4. Representative total ion chromatogram (TIC) of the pentane extract from the 3rd trap on HP-5MS column. Numbers refer to major compounds in Table 1.

methylphenols were 3-methylphenol (1.5–4.4%) and 4-methyl-2,6-bis(1,1-dimethylethyl)-phenol (3.4–4.5%), accompanied by minor percentages of other phenols (Table 1). Another group of identified phenols were methoxyphenols (major ones were 2-methoxyphenol (2.1–11.2%), 2-methoxy-4-methylphenol (3.4–7.7%) and 4-ethyl-2-methoxyphenol (2.9–3.1%)). Smoke derived phenolic compounds exhibit the low sensory threshold values (Rychlik, Schieberle, & Grosch, 1998) that make them important contributors to the "kulen" flavour. Eight derivatives of 2-cyclopenten-1-one, most likely originated from Maillard reactions of complex carbohydrate (cellulose) pyrolysis during smoking, were identified such as 3-methyl-2-cyclopenten-1-one (0.3–0.4%) or 2,3-dimethyl-2-cyclopenten-1-one (0.3–0.6%), Table 1. Among them, 3-ethyl-2-cyclopenten-1-one (ethyl cyclotene) exhibits a very strong odour and taste (Cutzach, Chatonnet, Henry, & Dubourdieu, 1997). The differences found in the concentration of smoke components among the samples could be due, among other causes, to being submitted to different smoking conditions.

The second cold trap containing 50 mL diethyl ether is mainly used for trapping the more volatile compounds that are not condensed earlier in the first trap. Representative chromatogram of ether extract volatiles from this trap is presented in Fig. 2. The majority of flavour important volatiles from this trap derived from spices added to the salami. Organosulphur compounds were identified including diallyl disulphide (20.6–31.2%), methyl allyl disulphide (0.6–2.1%) and diallyl trisulphide (0.0–0.4%) that can be connected with garlic origin and methional (0.0–0.4%). Namely, when garlic is chopped or crushed, allinase enzyme, present in garlic, is activated and acts on alliin to produce allicin (allyl 2-propenethiosulfinate) that has been established as olfactory and gustatory principle of fresh garlic. However allicin is unstable and decompose by several pathways (Kimbaris et al., 2006). According to one of them, it self-decomposes giving 2-propenesulfenic acid and thioacrolein (Fig. 3). The self-condensation of two molecules of thioacrolein yields two types of cyclic compounds 3-vinyl-(4H)-1,2-dithiin and 2-vinyl-(4H)-1,3-dithiin. These compounds can further decompose and form different sulphur compounds with general formula $R_1–(S)_n–R_2$. Among them, diallyl disulphide is probably direct product of allicin degradation or supersedes the synthesis of other polysulphides (Kimbaris et al., 2006) similar as diallyl trisulphide. Found methional (3-methylthiopropanal) is probably derived from methionine (Lee, Suriyaphan, & Cadwallader, 2001). Other ubiquitous spices derived compounds in this trap were terpenes, although with small percentages: limonene (0.4–1.0%), α-pinene (0.4–0.5%), δ-3-carene (0.0–0.3%) and β-ionone (0.0–0.2%). These results were expected since no spices with abundant terpene composition were used, such as black pepper, nutmeg, clove or others. A part of purged fatty acids, alcohols and aldehydes not condensed in the first trap was solved in diethyl ether of the second trap, such as hexadecanal (0.0–1.9%), 1-hexadecanol (1.2–7.9%), hexadecanoic acid (0.0–2.1%), (*Z*)-9-octadecen-1-ol (15.8–20.5%) and (*Z*)-9-octadecenoic acid (0.0–0.5%) as well as more volatile linear carbonyl compounds, Table 1. Phenols were also present and the major ones were phenol (0.6–1.6%), 2-methylphenol (0.2–1.2%), 2-methoxyphenol (2.0–2.1%), 2-methoxy-4-methylphenol (0.3–1.2%) and 4-methyl-2,6-bis(1,1-dimethyethyl)-phenol (6.0–7.1%). Several 2-cyclopenten-1-one derivatives from the first trap were also found such as 2-metyl-2-cyclopenten-1-one (0.0–0.5%), 3-methyl-2-cyclopenten-1-one (0.0–0.5%), 2-hydroxy-3-methyl-2-cyclopenten-1-one (0.0–0.6%) and 2,3-dimethyl-2-cyclopenten-1-one (0.0–0.4%).

The third cold trap, containing 50 mL of *n*-pentane is mainly used for trapping the compounds that escaped absorption in the first and second cold traps. One example of pentane extract chromatogram is presented in Fig. 4. Almost all condensed volatiles in this trap were already identified in previous two traps. Therefore this trap does not provide important contribution to the knowledge of "kulen" flavour. Principal constituents in this trap were

low-polar fatty acids and alcohols (*Z*)-9-octadecen-1-ol (23.8–32.4%), 1-hexadecanol (10.2–21.2%), hexadecanoic acid (1.2–5.9%) and 1-octadecanol (1.1–8.8%). Two organosulphur compounds were identified diallyl sulphide (0.0–1.6%) and diallyl disulphide (0.0–6.5%), of which the presence of the diallyl sulphide can be emphasised, since it was not found in previous traps (probably due to high volatility). Other isolated compounds with minor percentages were linear aliphatic and aromatic hydrocarbons Table 1.

3.1. General trends for comparison with other European salami volatiles

Reliable comparison of the obtained results with other European salami volatiles is very difficult due to strong impact of different isolation methods used in other studies (SD, SDE, headspace methods, HS-SPME and molecular distillation (MD)) to the qualitative and quantitative composition of isolated volatiles. For example, headspace and HS-SPME methods mainly collect compounds of low boiling points whereas losses of these compounds during application of NPSD method followed by concentration of the obtained ether and pentane extracts can be high (the absence of low boiling compounds is noticeable in Table 1). On the other hand, headspace and HS-SPME methods have limitations for quantitative isolation of medium volatile flavour compounds extracted by NPSD method with different separation among the traps (fractionation according to volatility and solubility). Therefore the aim of further comparison is to indicate possible trends of "kulen" volatile flavour compounds that could be used for discrimination among other European salami volatiles.

The large analytical differences among four salami brands from France "Aoste", Italy "italsalumi", Spain "gran serrano" and Germany "Giesike" were examined (Schmidt & Berger, 1998) after lipid extraction and molecular distillation (MD). MD generally gave good yields over the entire spectrum of volatiles. Terpenes (limonene, α-pinene, β-pinene, δ-3-carene, β-caryophyllene, γ-terpinene and others) and fatty acids up to C10 dominated, followed by aliphatic alcohols (such as 2,3-butanediol) and ketones (particularly 2-butanone and 2-hydroxy-2-butanone). Esters and sulphur compounds (major ones diallyl disulphide and diallyl sulphide) were the main minor constituents. Very high concentrations of safrole were found in the French and German salami as well as similar amounts of the structurally related myristicine. High contents of hexanal of oxidative origin, ethanol and acetone from fermentative origin were found by static headspace sampling of different Italian salami ("Milano", "Felino", "Filzetta", "Cacciatorino", "Campagnolo", "Soppressa" and "Piccante" with capsicum) whilst only carbon disulphide was identified among organosulphur compounds and no phenols were found (Procida, Conte, Fiorasi, Comi, & Gabrielli Favretto, 1999). Dynamic headspace volatile concentration of "Milano" salami isolated high content of terpenes, low content of diallyl disulphide and 1-propene-3-methylthio as the major aliphatic sulphur compound (Meynier, Novelli, Chizzolini, Zanardi, & Gandemera, 1999). Spices origin terpenes were predominated in a typical Sicilian salami (Moretti et al., 2004) isolated by simultaneous distillation–extraction (SDE). In order to characterise two kinds of typical Italian dry-sausages, namely "Salame Mantovano" and "Salame Cremonese", their volatile composition was determined by the dynamic headspace extraction technique (DHS) coupled with gas chromatography–mass spectrometry (GC–MS). The data obtained shows that the most important contributions to the differentiation of the two kinds of typical Italian salami were 3-methylbutanal, 6-camphenol, dimethyl disulphide, 1-propene-3,3′-thiobis, ethyl propanoate, 1,4-*p*-menthadiene and 2,6-dimethyl-1,3,5,7-octatetraene (Bianchi et al., 2007).

By comparing the above results with Table 1 the following discrimination trends of "kulen" volatiles can be pointed out: (1) lower amount of terpenes (only α-pinene, δ-3-carene, limonene and β-ionone were found among "kulen" volatiles) whilst terpenes were one of the most abundant class of aroma compounds identified in different European salami; (2) the high percentages of methoxyphenols and methylphenols (such as phenol, 2-methylphenol, 3-methylphenol, 2-methoxyphenol, 2-methoxy-4-methylphenol, 4-ethyl-2-methoxyphenol and 4-methyl-2,6-bis(1,1-dimethylethyl)phenol) as well as 2-cyclopenten-1-one derivatives (such as ethyl cyclotene) can be emphasised since smoking is not ubiquitous in many European salami; (3) presence of high percentages of diallyl disulphide with minor percentages of other organosulphur compounds; (4) relatively low percentages of low-molecular lipid oxidation carbonyl compounds and alcohols (lower than C7) that were probably lost during NPSD isolation.

4. Conclusions

NPSD headspace volatile compounds from lipid oxidation, amino acid degradation, smoke and spices have been isolated and identified with different distribution among the traps that enabled comprehensive volatile profiling. The volatiles from first two traps were the most representative for "kulen" profiling. The major identified flavour important compounds were methylphenols, methoxyphenols, organosulphur compounds (diallyl disulphide, methylallyl disulphide, diallyl trisulphide, diallyl sulphide and methional) and some derivatives of 2-cyclopenten-1-one such as ethyl cyclotene. Non-important flavour constituents such as fatty acids, alcohols and aldehydes were also present among abundant compounds. General trends for comparison with other European salami volatiles were noticed by NPSD as the consequence of smoke treatment, and added spices, less related to the type of meat and long period of ripening. The same isolation method should be used in further research fore more reliable comparison with other salami volatiles providing a more precise set of discriminating and/or similarity parameters.

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