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Volatile aroma compounds in Moskovskaya cooked smoked sausage formed in different types of casings

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Abstract: The paper presents a study of Moskovskaya cooked smoked sausages formed in various artificial casings: fibrous (cellulose), collagen, and polyamide. An oxygen permeability oxygen permeability of the casings was above 40 cm^3 and below 30 cm^3/m^2·24 h·bar. The study involved a sensory evaluation and instrumental tests using a VOCmeter multi-sensor system ('electronic nose') and a 7890A gas chromatograph with a 5975C VLMSD mass-selective detector (Agilent Technologies). We obtained original data on the qualitative composition and the quantitative content of substances that form the aroma of cooked smoked sausages in various types of casings. We found that the samples contained two groups of compounds with the chemical formulas of $C_iH_kO_l$ and $C_iH_kO_lN_m$. They had a ratio of (12–33):1 and were, apparently, the most significant aromatic substances. The main class of identified compounds was carboxylic acid esters, accounting for 76.61–81.60% of the total substances. We established a correlation between the aroma intensity and the concentration of chlorine-containing and nitrogen-containing compounds (except amines, amides, nitriles, and hydrazides) in the gas phase. The results did not confirm our hypothesis about the influence of the casing type and its permeability on the development of oxidative processes in the production of cooked smoked sausages. The practical significance of the study lies in creating a database of over 200 aromatic compounds that allows for a deeper understanding of aroma formation processes in cooked smoked sausages. The database can be used to exert a purposeful technological influence on the quality indicators and create various flavour compositions to adjust the sensory properties of the product.

Keywords: Flavours of meat products, sensory evaluation, 'electronic nose', gas chromatographic analysis, artificial casings

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INTRODUCTION

In recent decades, sausage producers have significantly expanded the range of casings for cooked, smoked, and cooked smoked sausages. The latter are highly popular, especially in the summer, due to their sensory characteristics, high nutritional value, long shelf-life, and a relatively low price compared to expensive dry sausages [1, 2]. Aroma is one of the key factors of consumer preference [3–9]. The classical technology of making cooked smoked sausages involves a fairly long cooking process that includes boiling, cooling, smoking (one or two stages), and drying. Such a process demands using only permeable casings [10–12]. Artificial casings made of collagen, cellulose, and polyamide are widely used by modern producers for various reasons. Some of them include standard characteristics of steam and gas permeability, as well as geometrical dimensions, which allow for automatic sausage forming [13]. Growing competition forces sausage producers to focus on technology, rather than the price or outcome, when choosing casings. In particular, they look at the effect that technology has on the product's sensory characteristics [14]. In this regard, of great scientific and practical interest is a study that aims to objectively assess the composition of volatile substances in the aroma of cooked smoked sausages formed in various types of artificial casings.

STUDY OBJECTS AND METHODS

Our objects of study were samples of Moskovskaya cooked smoked sausage (whole sausages) produced by the same shift on the same day according to State Standard R 55455-2013. Boiled-smoked meat sausages. Specifications*. The sausages were formed in the following casings: sample no. 1 in a fibrous (cellulose) casing, sample no. 2 in a collagen casing, sample no. 3 in a highly permeable polyamide with an oxygen permeability above 40 $cm^3/m^2 \cdot 24$ h·bar, and sample 4 in a permeable polyamide casing with an oxygen permeability less than 30 $cm^3/m^2 \cdot 24$ h·bar.

All the samples were produced at a sausage factory. After cooling, they were packed in impermeable bags to preserve their aroma and sent to V.M. Gorbatov Federal Research Centre for Food Systems.

The sensory evaluation of sausages was carried out according to State Standard 9959-2015**. The taste panel consisted of 7 qualified experts. The results were confirmed by instrumental sensory data produced by the VOCmeter (‘electronic nose’). The device is equipped with highly sensitive nanosensors capable of capturing volatile components released from the surface of the product. Prior to testing, the sausages were crushed and at least three 3 g samples were taken from each of them. The samples were placed in special vials and sealed. The vials were alternately placed into the chamber, where each sample was heated to 50°C. Then, the lid of the vial was punctured with a needle, and the gas phase was taken from near the sample surface. The gas phase entered the surface of the nanosensors sensitive to various classes of chemical compounds. Any physicochemical changes that occurred on the surface of the nanosensors were converted into an electronic signal, transmitted to a computer, and statistically processed by the software. We used four metal oxide nanosensors (M1–M4) sensitive to those aroma-producing volatile substances which are characteristic of meat products. They include products of protein breakdown, fat oxidation, ketones, aldehydes, volatile fatty acids, ammonia and other substances [15–16].

The composition of volatile aroma components was analysed by a 7890A gas chromatograph with a 5975C VLMSD mass-selective detector (Agilent Technologies, USA). For this, volatile substances were preliminarily extracted (1:1) with 40% aqueous ethanol and chloroform-methanol according to the Folch method, followed by methylation with a solution of acetyl chloride in methanol. The composition of aroma components was determined by a HP-5MS capillary column with a diameter of 0.25 mm, a length of 30 m, and a stationary phase layer thickness of 0.25 µm.

The chromatography was carried out under the following conditions:
– carrier gas: He;
– flow rate: 1 ml/min;
– injector temperature in a no-split mode: 250°C;
– initial temperature of the column thermostat: 100°C for 2 minutes;
– programmable heating from 100°C to 290°C at a rate of 20°C/min;
– an isotherm at 290°C: up to 25 min; and
– component analysis duration: 25 min.

The identification parameters were as follows:
– ion source temperature: 230°C;
– quadrupole temperature: 150°C;
– electron energy: 70 eV;
– scan mode: full; and
– mass range: 33–1050 amu.

The peaks were analysed using the NIST08 MS Library, an automated search and identification database, and the substances were named according to the IUPAC. The analysis covered those substances whose mass content in the mixture of volatile compounds exceeded 0.01%. The probability of peak correlation had to be at least 35% [17].

RESULTS AND DISCUSSION

The sensory evaluation of the Moskovskaya sausage samples in various casings did not reveal any significant differences in their consistency, colour, taste, or aroma. The tasters noted a more pronounced smoking aroma in samples no. 2 and 3, compared to samples no. 1 and 4, and a firmer surface layer in samples no. 1 and 2. They did not establish any differences in taste and aroma between samples no. 1, 2, and 3; however, they found them less pronounced in sample no. 4.

The ‘electronic nose’ was used to quantitatively identify the minimum differences in the gas phase aroma (Fig. 1).

The highly sensitive nanosensors revealed no significant differences in aroma between the samples. This was evidenced by the general nature of nanosensor responses, with the strongest signal coming from M4 and M2. Moreover, there was an image resembling a geometric figure and no intersection between the lines connecting the scale points that corresponded to the

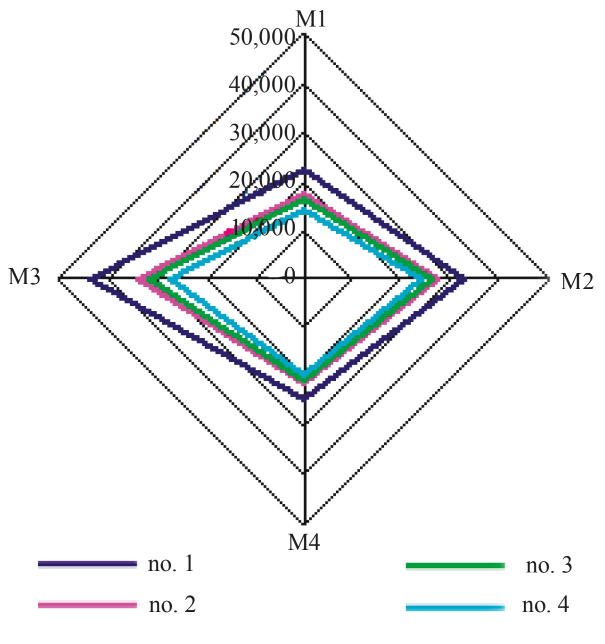

Fig. 1. Multisensory aroma profiles of Moskovskaya sausage samples produced by the ‘electronic nose’.

*State Standard R 55455-2013. Boiled-smoked meat sausages. Specifications. Moscow: Standartinform Publ., 2014. 14 p.

**State Standard 9959-2015. Meat and meat products. General conditions of organoleptical assessment. Moscow: Standartinform Publ., 2016. 20 p.

signals of the four nanosensors. The multisensory profiles of samples no. 2 and 3 practically coincided, indeed.

The analysis of sample no. 1 showed stronger signals coming from M2 and M4. These nanosensors are sensitive to the presence of aldehydes, ketones, and heterocyclic aromatic compounds in the gas phase. This might result from more intensive oxidative processes and/or an increased concentration of volatile substances due to a rapid loss of moisture during heat treatment. Another reason might be a more intensive accumulation of substances that enter the product through the casing during smoking.

Table 1. Identification and analysis of major volatile substances in Moskovskaya sausage formed in a fibrous casing (sample no. 1)

Peak no.	Time, min	Mass scanning			Peak height, cu	Peak area, cu	Content, % of max. peak	Content, % of amount	Substance	Probability of peak identification for standard mass spectrum, %
		Start	Max.	Finish						
1	2	3	4	5	6	7	8	9	10	11
2	8.196	862	869	880	1,582	2,171	0.20	0.10	4,6-Dimethyl-2-thioxo-1,2-dihydro-3-pyridinecarbonitrile	50
3	8.300	880	889	904	854	2,101	0.19	0.10	4-Acetamido-N,N-diisobutyl-3-nitrobenzamide	50
4	9.841	1,171	1,186	1,195	773	1,870	0.17	0.09	2-Pyrroline-3-carboxylic acid, 4-(4-chlorobenzylidene)-2-methyl-5-oxo-, methyl ester	46
5	10.884	1,375	1,387	1,399	3,141	4,046	0.37	0.19	2-Chloro-N-(1-m-tolyl-2,3-dihydro-1H-pyrrolo[2,3-b]quinolin-4-yl)-acetamide	38
6	12.160	1,621	1,633	1,642	2,296	3,677	0.34	0.17	3-Bromo-N'-(1-(2-thienyl)ethylidene)benzohydrazide	80
7	12.243	1,642	1,649	1,660	10,315	11,495	1.06	0.54	Methyltetradecanoate	87
8	12.383	1,660	1,676	1,687	33,372	33,679	3.10	1.57	2H-1-Benzopyran-2-one, 7-(4-methyl-5-phenyl-2H-1,2,3-triazol-2-yl)-3-phenyl-	64
9	13.519	1,879	1,895	1,909	14,416	21,256	1.96	0.99	9-Hexadecenoic acid, methyl ester, (Z)-	50
10	13.665	1,909	1,923	1,948	233,070	255,890	23.54	11.92	Pentadecanoic acid, 14-methyl-, methyl ester	95
11	13.960	1,963	1,980	1,996	7,701	9,879	0.91	0.46	Ether, methyl 1-tetradecenyl	50
12	14.163	2,005	2,019	2,035	1,730	3,353	0.31	0.16	10-Undecynoic acid, methyl ester	52
13	14.292	2,035	2,044	2,056	1,941	2,571	0.24	0.12	Heneicosanoicacid, methyl ester	50
14	14.443	2,056	2,073	2,089	1,233	3,928	0.36	0.18	Hydrazine, 1,1-diethyl-2-(1-methylethyl)-	47
15	14.817	2,119	2,145	2,158	605,268	1,087,059	100.00	50.65	9-Octadecenoic acid (Z)-, methyl ester	99
16	14.936	2,158	2,168	2,185	131,811	155,923	14.34	7.26	Octadecanoic acid, methyl ester	98
17	15.055	2,185	2,191	2,197	1,997	4,288	0.39	0.20	Ethanol, 2-[(2-ethylhexyl)oxy]-	91
18	15.112	2,197	2,202	2,212	2,377	4,235	0.39	0.20	Silane, triethyl-2-pentenyl-, (Z)-	38
19	15.200	2,212	2,219	2,233	6,390	9,057	0.83	0.42	Octadec-9-en-1-al dimethyl acetal	53
20	15.335	2,236	2,245	2,269	516	2,632	0.24	0.12	Acetamide, N-(4-hydroxycyclohexyl)-, trans-	37
22	15.834	2,335	2,341	2,347	4,034	7,132	0.66	0.33	1-Chlorosulfonyl-3-methyl-1-azaspiro[3.5] nonan-2-one	80
23	15.942	2,347	2,362	2,383	60,559	104,085	9.57	4.85	10-Undecenoyl chloride	43
24	16.083	2,383	2,389	2,407	2,145	7,197	0.66	0.34	Pentanoic acid, methylester	35
25	16.492	2,461	2,468	2,479	766	1,853	0.17	0.09	1,2-Ethanediamine, N,N,N'-trichloro-N',1,1,2,2-pentafluoro-	47
26	16.658	2,482	2,500	2,509	843	1,893	0.17	0.09	Cyclohexasiloxane, dodecamethyl-	37
28	17.042	2,569	2,574	2,617	36,177	71,967	6.62	3.35	2-Methyl-3,4,5,6-tetrahydropyrazin	84
30	18.692	2,884	2,892	2,911	825	2,221	0.20	0.10	Perhydro-htx-2-one, 2-depentyl-, acetate ester	38
32	19.383	3,016	3,025	3,034	699	1,891	0.17	0.09	5H-Cyclopropa[3,4]benz[1,2-e] azulen-5-one, 9,9a-bis(acetyloxy)-1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4	43
39	22.631	3,640	3,651	3,670	4,500	16,866	1.55	0.79	Ledeneoxide-(II)	38
40	22.869	3,691	3,697	3,712	553	1,697	0.16	0.08	4-(3,4-Methylenedioxyphenyl)-2-butanone	46
41	23.035	3,718	3,729	3,736	757	1,644	0.15	0.08	Silanamine, N-[2,6-dimethyl-4-[(trimethylsilyl)oxy] phenyl]-1,1,1-trimethyl-	43
43	23.705	3,847	3,858	3,862	664	1,689	0.16	0.08	N-Methyl-1-adamantaneacetamide	35
44	23.954	3,898	3,906	3,922	583	2,230	0.21	0.10	2,4-Di-tert-butyl-6-(tert-butylamino)phenol	37
47	24.332	3,970	3,979	3,988	823	2,468	0.23	0.12	11H-Dibenzo[b,e][1,4]diazepin-11-one, 5,10-dihydro-5-[3-(methylamino)propyl]-	49

Table 2. Identification and analysis of major volatile substances in Moskovskaya sausage formed in a collagen casing (sample no. 2)

Peak no.	Time, min	Mass scanning			Peak height, cu	Peak area, cu	Content, % of max. peak	Content, % of amount	Substance	Probability of peak identification for standard mass spectrum, %
		Start	Max.	Finish						
1	2	3	4	5	6	7	8	9	10	11
2	4.040	58	68	82	540	2,314	0.37	0.18	Butyric acid, 4-(4-chloro-5-methyl-3-nitro-pyrazol-1-yl)-	35
5	8.196	853	869	877	903	1,803	0.29	0.14	Indolizine, 6-ethyl-2-phenyl-	47
6	8.300	877	889	898	1,422	2,272	0.37	0.18	2,3,4-Trimethoxyphenylacetonitrile	64
7	9.841	1,168	1,186	1,195	885	2,408	0.39	0.19	[5-[(Furan-2-carbonyl)amino]-3-methylpyrazol-1-yl]acetic acid, ethyl ester	60
8	10.878	1,357	1,386	1,405	2,961	5,092	0.83	0.40	p-Pentyloxybenzylidene p-hexylaniline	53
9	12.155	1,603	1,632	1,642	2,568	4,091	0.66	0.32	Benzaldehyde, 2-(2-phenoxyethoxy)-, 1-cyclohexylsemicarbazone	45
10	12.243	1,642	1,649	1,657	6,230	6,647	1.08	0.52	Pentanoic acid, 4-methyl-, methyl ester	72
11	12.388	1,663	1,677	1,687	64,541	61,950	10.04	4.81	2H-1-Benzopyran-2-one, 7-(4-methy-5-phenyl-2H-1,2,3-triazol-2-yl)-3-phenyl-	72
12	13.519	1,882	1,895	1,909	7,860	12,314	2.00	0.96	9-Octadecenoic acid (Z)-, methyl ester	53
13	13.659	1,909	1,922	1,936	125,948	139,968	22.68	10.87	Hexadecanoic acid, methylester	95
14	13.955	1,960	1,979	1,993	6,428	9,077	1.47	0.71	Butanoic acid, 2-hexenyl ester, (E)-	50
16	14.292	2,026	2,044	2,050	1,217	2,335	0.38	0.18	Undecanoic acid, methyl ester	64
17	14.443	2,065	2,073	2,086	1,732	3,725	0.60	0.29	Ethane, isothiocyanato-	43
19	14.801	2,116	2,142	2,158	367,029	617,113	100.00	47.91	9-Octadecenoic acid (Z)-, methyl ester	99
20	14.931	2,158	2,167	2,182	74,923	96,174	15.58	7.47	Octadecanoicacid, methyl ester	94
22	15.112	2,194	2,202	2,209	3,697	4,520	0.73	0.35	Silane, triethyl-2-pentenyl-, (Z)-	50
23	15.195	2,209	2,218	2,227	5,201	6,583	1.07	0.51	1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-	43
24	15.740	2,314	2,323	2,335	5,643	8,862	1.44	0.69	4-Hexadecen-6-yne, (E)-	53
26	15.932	2,350	2,360	2,383	30,041	46,693	7.57	3.63	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	46
27	16.077	2,383	2,388	2,404	1,063	2,428	0.39	0.19	Dodecanoic acid, 2-methyl-	52
29	16.767	2,509	2,521	2,527	1,697	2,520	0.41	0.20	Silane, triethyl-2-pentenyl-, (Z)-	72
30	16.923	2,533	2,551	2,563	49,234	103,407	16.76	8.03	9-Oxabicyclo[6.1.0]nonane	86
31	17.032	2,563	2,572	2,611	14,819	44,786	7.26	3.48	Oxalic acid, isobutyl tridecyl ester	35
37	18.983	2,944	2,948	2,962	553	1,810	0.29	0.14	trans-2,3-Methylenedioxy-b-methyl-b-nitrostyrene	53
38	19.180	2,977	2,986	3,013	1,353	6,405	1.04	0.50	4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-.alpha.-methyl-, methyl ester	38
39	19.585	3,055	3,064	3,082	669	2,209	0.36	0.17	4,6-Bis(diethylamino)-1,3,5-triazine-2-carbonylhydrazide	43
44	21.515	3,421	3,436	3,442	978	3,758	0.61	0.29	N-Methyl-1-adamantaneacetamide	40

The statistical processing of the nanosensor signals showed the following multisensory profile areas that characterized the intensity of the samples' aroma ($S \cdot 10^7$, cu, $P > 0.95$): 179.06; 118.91; 106.51; and 84.87 for samples no. 1, 2, 3, and 4, respectively. Thus, if we take the aroma intensity of sample no. 4 (minimum value) as 100%, the intensity of samples no. 1, 2, and 3 was 211%, 140%, and 125%, respectively. These differences indicated a need for further analysis of volatile substances.

It is noteworthy that it was the first study into the composition of volatile components in cooked smoked sausages. The most studied aroma is that of fermented raw and dry sausages [3–6]. Moskovskaya cooked smoked sausage is only made of beef and fatback, as well as a nitrite-curing mixture, sugar, and spices (black pepper, cardamom or nutmeg). Therefore, it was an excellent model for studying aroma in this type of meat products.

Tables 1–4 present the identification and statistical processing results for volatile substances in the sausage samples obtained with the gas chromatograph software and the automated search and identification database [22].

We used the NIST08 MS Library automated database to identify volatile substances with a peak correlation probability of more than 35%. Of total volatile substances, we identified 85.9; 93.31; 94.43; and 93.72% of substances in samples no. 1, 2, 3, and 4, respectively. These amounts corresponded to the peaks presented in Tables 1–4.

The atomic composition of the identified volatile substances contained 10 elements from Mendeleev's Periodic Table, including hydrogen, carbon, oxygen, and nitrogen. These elements are the most typical in

Table 3. Identification and analysis of major volatile substances in Moskovskaya sausage formed in a highly permeable polyamide casing (sample no. 3)

Peak no.	Time, min	Mass scanning			Peak height, cu	Peak area, cu	Content, % of max. peak	Content, % of amount	Substance	Probability of peak identification for standard mass spectrum, %
		Start	Max.	Finish						
1	2	3	4	5	6	7	8	9	10	11
2	4.128	76	85	103	410	1,452	0.30	0.15	Iron, (2-formyl norbornadiene)tricarbonyl	35
3	10.883	1,372	1,387	1,396	817	1,518	0.31	0.15	1,3-Dimethyl-7-O-tolyl-5,5-bis-trifluoromethyl-5,8-dihydro-1H-pyrimido[4,5-d] pyrimidine-2,4-dione	45
5	12.238	1,642	1,648	1,660	3,520	4,767	0.97	0.48	Nonanoic acid, methyl ester	59
6	12.378	1,660	1,675	1,684	9204	11,033	2.24	1.12	1,2,3,4-Tetrahydroisoquinolin-6,7-diol, 1-phenylmethylene-, 2,6,7-triacetate	59
7	13.514	1,876	1,894	1,903	5,305	6,745	1.37	0.69	4-Nonenoic acid, methyl ester	42
8	13.654	1,909	1,921	1,948	111,673	120,593	24.53	12.25	Tridecanoic acid, methyl ester	97
12	14.796	2,119	2,141	2,158	300,232	491,592	100.00	49.92	9-Octadecenoic acid, methyl ester, (E)-	99
13	14.925	2,158	2,166	2,185	79,623	90,670	18.44	9.21	Octadecanoic acid, methyl ester	97
15	15.195	2,209	2,218	2,230	1,689	2,793	0.57	0.28	Pentanoic acid, 5,5-dimethoxy-, methyl ester	50
18	15.735	2,311	2,322	2,329	2,962	4,410	0,90	0.45	Methyl 3-hydroxyoctadec-9-enoate	74
20	15.927	2,350	2,359	2,383	25,335	41,069	8.35	4.17	15-Hydroxypentadecanoic acid	50
21	16.077	2,383	2,388	2,401	1,211	2,480	0.50	0.25	Methyl 18-methylnonadecanoate	43
24	16.923	2,530	2,551	2,566	43,792	92,020	18.72	9.34	9,17-Octadecadienal, (Z)-	42
25	17.032	2,566	2,572	2,632	12,672	32,128	6.54	3.26	Undecanoylchloride	35
27	18.371	2,827	2,830	2,851	749	3,101	0.63	0.32	Phenol, 4-[2-(5-nitro-2-benzoxazolyl) ethenyl]-	43
29	18.692	2,869	2,892	2,893	764	3,392	0.69	0.34	Alanine, 3,3,3-trifluoro-2-[(4-methoxybenzoyl) amino]-N-[3-(trifluoromethyl)-2-quinoxalinyl]-, ethyl ester	38
31	18.843	2,911	2,921	2,929	680	2,007	0.41	0.20	1,2-Benzenediol, O,O'-di(propargyloxycarbonyl)-	35
36	19.408	3,025	3,030	3,040	811	1,638	0.33	0.17	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl nonyl ester	35
42	20.84	3,298	3,306	3,313	556	1,507	0.31	0.15	1,2-Bis(trimethylsilyl)-3,6-dimethylcyclohexane-1,4-diene	47
43	21.224	3,373	3,380	3,388	703	1,999	0.41	0.20	2,6-Naphthalenediol, 1,5-bis[(piperonylimino)methyl]-	35
46	22.605	3,634	3,646	3,670	2,024	9,306	1.89	0.95	Benzamide, 3-methoxy-N-[4-(1-methylcyclopropyl) phenyl]-	43
48	23.29	3,769	3,778	3,790	558	1,665	0.34	0.17	Benzamide, N-(1,1-dimethylethyl)-4-methoxy-	47

products of animal and plant origin with a cellular structure. Also present were chlorine, sulphur, silicon, fluorine, bromine, and iron (Table 5).

The presence of organosilicon compounds was due to the use of a capillary column based on (5%-phenyl)-methylpolysiloxane. This group of compounds accounted for 0.36% to 0.64% of total volatile substances. Due to their origin and insignificant amount, they were excluded from further analysis.

As can be seen from Table 5, all the studied samples contained two groups of compounds with the general chemical formulas of $C_iH_kO_l$ and $C_iH_kO_lN_m$. Apparently, they were the most significant compounds in the aroma of Moskovskaya sausage. Their content was 33:1, 12:1, 32:1, and 25:1 in samples no. 1, 2, 3, and 4, respectively, which could be summarized as 12–33:1.

The greatest variety of compounds was found in sample no. 1 (fibrous casing) and sample no. 4 (permeable polyamide casing). The total amount of oxygen-containing compounds was slightly higher in sample no. 3 (highly permeable polyamide casing) than in sample no. 4 (polyamide casing with lower permeability). At the same time, the content of oxygen-containing compounds in sample 1 (fibrous casing) was 11.88% (absolute value) lower than in sample no. 3. Thus, the formation of a significant amount of oxygen-containing substances in the gas phase of a product could not be explained by the choice of casing or its degree of permeability.

Table 6 shows the content of volatile substances belonging to different classes of chemical compounds. As can be seen, carboxylic acid esters were the main

Table 4. Identification and analysis of major volatile substances in Moskovskaya sausage formed in a permeable polyamide casing (sample no. 4)

Peak no.	Time, min	Mass scanning			Peak height, cu	Peak area, cu	Content, % of max. peak	Content, % of amount	Substance	Probability of peak identification for standard mass spectrum, %
		Start	Max.	Finish						
1	2	3	4	5	6	7	8	9	10	11
1	3.868	28	35	61	1,153	3,970	0.28	0.16	Benzeneethanamine, N-[(pentafluorophenyl)methylene]-4-[(trimethylsilyl) oxy]-	35
3	8.196	859	869	877	1,998	2,381	0.17	0.10	4,6-Dimethyl-2-thioxo-1,2-dihydro-3-pyridinecarbonitrile	50
4	8.3	883	889	904	1,594	2,321	0.17	0.09	2-Methyl-7-phenylindole	47
5	8.907	985	1,006	1,021	851	2,086	0.15	0.08	10-Undecynoic acid, methyl ester	72
6	9.846	1,171	1,187	1,201	580	1,671	0.12	0.07	1,2-Dihydroindeno[1,2,3-cd]pyrene	37
7	10.884	1,378	1,387	1,402	3,894	4,724	0.34	0.19	(6-Phenylsulfanyl-5-trifluoromethylpyridin-3-yl)carbamic acid, prop-2-ynyl ester	50
8	11.408	1,474	1,488	1,501	624	1,748	0.12	0.07	2-p-Chlorophenyl-6,8-dimethyl-4-[1,2-epoxy-2-propyl]quinoline	35
10	12.248	1,639	1,650	1,663	12,357	14,835	1.06	0.59	Tridecanoic acid, 12-methyl-, methyl ester	83
11	12.388	1,663	1,677	1,690	90,045	84,605	6.04	3.37	2H-1-Benzopyran-2-one, 7-(4-methyl-5-phenyl-2H-1,2,3-triazol-2-yl)-3-phenyl-	59
12	12.959	1,768	1,787	1,795	807	2,070	0.15	0.08	Cyclopentanetridecanoicacid, methyl ester	53
13	13.519	1,876	1,895	1,903	18,196	25,427	1.82	1.01	9-Octadecenoic acid (Z)-, methyl ester	95
14	13.67	1,909	1,924	1,948	270,802	327,084	23.36	13.03	Pentadecanoic acid, 14-methyl-, methyl ester	97
15	13.96	1,963	1,980	1,990	6,884	10,126	0.72	0.40	trans-2-Decen-1-ol, methyl ether	50
16	14.158	2,005	2,018	2,026	1,961	3,616	0.26	0.14	2,4,3,5-Diethylidene-l-xylose	50
17	14.292	2,038	2,044	2,056	2,635	3,243	0.23	0.13	Dodecanoic acid, methylester	64
19	14.817	2,110	2,145	2,161	749,047	1,400,183	100.00	55.78	9-Octadecenoic acid (Z)-, methyl ester	99
20	14.936	2,161	2,168	2,182	137,725	172,630	12.33	6.88	Octadecanoicacid, methyl ester	97
21	15.05	2,182	2,190	2,194	1,853	4,677	0.33	0.19	Cyclopropanenonanoic acid, methyl ester	47
22	15.117	2,194	2,203	2,212	3,839	6,142	0.44	0.25	Silane, triethyl-2-pentenyl-, (Z)-	50
23	15.2	2,212	2,219	2,242	6,331	8,829	0.63	0.35	Phytol	38
24	15.745	2,317	2,324	2,335	6,674	10,928	0.78	0.44	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	64
25	15.828	2,335	2,340	2,350	3,107	6,316	0.45	0.25	4-(6-Methyl-4-methylene-3,4,5,6-tetrahydro-2H-pyran-2-yl)-1-butanol	38
27	16.083	2,383	2,389	2,401	1,515	2,373	0.17	0.10	Pentanoicacid, methyl ester	58
31	16.933	2,530	2,553	2,563	96,726	174,952	12.49	6.97	9-Oxabicyclo[6.1.0]nonane	86
32	17.037	2,563	2,573	2,605	26,231	67,728	4.84	2.70	Eicosanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	38
35	17,909	2,725	2,741	2,743	953	2,876	0.21	0.12	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	37
45	22,158	3,556	3,560	3,580	663	2,067	0.15	0.08	Benzamide, 4-methoxy-N-[4-(1-methylcyclopropyl) phenyl]-	35

class of identified compounds in all the samples. Their mass fraction in the total amount of identified substances ranged from 76.61% to 81.60%. Another 4 classes of compounds, present in all the samples, were represented less evenly. For example, the content of alcohols, oxygen-containing heterocycles (except ketones and aldehydes), and nitrogen-containing heterocycles (except heterocyclic amines, amides and hydrazides) ranged from 0.3% to 0.51%, 0.2% to 8.03%, and 0.26% to 5.02%, respectively.

A detailed analysis of the classes of substances present in the aroma of the samples, as well as their elemental analysis, did not reveal any relationship between the type and permeability of the casing and the characteristics of volatile substances.

Carboxylic acid esters were mainly represented by methyl esters and less frequently by ethyl esters. On the one hand, this could be explained by the sample preparation method using methylation. On the other hand, methyl and ethyl esters could already be present in the product during its manufacture. The esters identified in the samples differed in their molecular weight, chain length, and the presence of not only carbon, hydrogen, and oxygen, but also nitrogen, chlorine, and fluorine.

Table 5. Atomic composition of volatile compounds in Moskovskaya sausage aroma

Chemical formula of identified compounds	Content of identified compounds, % of total amount			
	Sample			
	no. 1	no. 2	no. 3	no. 4
1	2	3	4	5
C_iH_k	–	0.69	–	0.07
$C_iH_kN_l$	3.35	0.14	–	0.09
$C_iH_kN_lF_mCl_n$	0.09	–	–	–
$C_iH_kN_lS_m$	–	0.29	–	–
$C_iH_kN_lS_mSi_n$	0.10	–	–	0.10
$C_iH_kO_l$	74.12	84.46	87.41	89.12
$C_iH_k O_lCl_m$	4.85	–	3.26	–
$C_iH_kO_lN_m$	2.27	7.00	2.76	3.57
$C_iH_kO_lN_mBr_nS_pSi_q$	0.17	–	–	–
$C_iH_kO_lN_mCl_n$	0.28	0.18	–	0.07
$C_iH_kO_lN_mF_n$	–	–	0.49	–
$C_iH_kO_lN_mF_nSi_p$	–	–	–	0.16
$C_iH_kO_lN_mS_nCl_p$	0.33	–	–	–
$C_iH_kO_lN_mS_nF_p$	–	–	–	0.19
$C_iH_kO_lN_m Si_n$	0.08	–	–	–
$C_iH_kO_lS_m$	–	–		0.10
$C_iH_kO_lSi_m$	0.09	–	–	–
$C_iH_kO_lFe_m$	–	–	0.15	–
$C_iH_kSi_l$	0.20	0.55	0.36	0.25
Total identified compounds	85.93	93.31	94.43	93.72
Including compounds containing:				
- oxygen	82.19	91.64	94.07	93.21
- nitrogen	6.67	7.61	3.25	4.18
- chlorine	5.55	0.18	3.26	0.07
- sulphur	0.60	0.29	–	0.39
- silicon	0.64	0.55	0.36	0.51
- fluorine	0.09	–	0.49	0.35

In total, we found over 35 compounds with a number of carbon atoms from 6 to 23. The most represented in all the samples were the methyl esters of oleic acid with the number of carbon atoms C_{19} (Table 7). The predominance of this ester was due to the fatty acid composition of fatback: the content of this monounsaturated acid ranged from 30% to 45% of the total fatty acids.

We were mostly interested in those groups of substances which were found in all Moskovskaya sausage samples as a result of the sensory evaluation and the "electronic nose" tests. The data allowed us to check our hypothesis about a correlation between the aroma intensity established by the "electronic nose" and the total content of substances in the gas phase of the samples (Table 8).

The correlation analysis produced an unexpected result: an increase in the aroma intensity was proportional to the increase in the content of nitrogen-containing heterocycles and chlorine-containing substances. In that case, it was reasonable to consider only positive values of the correlation coefficients, since the hypothesis that the nanosensor signals increased as the concentration of certain substances decreased had no physical sense.

Table 6. Major classes of chemical compounds in Moskovskaya sausage aroma

Class	Content of compounds by class, % of total amount			
	Sample			
	no. 1	no. 2	no. 3	no. 4
1	2	3	4	5
Hydrocarbons				
alkanes	–	0.98	–	–
arenes	–	0.14	–	0.07
Oxygen-containing				
alcohols	0.3	0.51	0.32	0.45
aldehydes	–	–	9.34	–
carboxylic acid	–	0.18	4.17	–
esters	77.9	76.61	77.5	81.60
heterocyclic aldehydes (including nitrogen-containing)	-	0.32		0.14
heterocyclic ketones (including nitrogen-containing)	0.62		0.15	3.37
other oxygen-containing heterocycles	0.79	8.03	0.2	7.22
Nitrogen-containing				
amines	0.09	0.4	–	0.12
amides	0.08	0.29	–	–
hydrazines	0.18	–	–	–
nitriles	–	0.18	–	–
heterocyclic amines	–	–	–	0.16
heterocyclic amides	0.41	–	1.12	0.08
heterocyclic hydrazides	0.17	0.17	–	–
other nitrogen-containing heterocycles	5.02	4.95	1.12	0.26
Iron-containing				
heterocycles	–	–	0.15	–
Total (without silicon-containing compounds)	85.29	92.76	94.07	93.21

CONCLUSION

The study produced original data on the qualitative composition and the quantitative content of substances that form the aroma of Moskovskaya cooked smoked sausage. It involved a detailed comparative analysis of the main classes of compounds present in the gas phase of the samples formed in various types of casings. We found that all the samples contained two groups of compounds with the general chemical formulas of $C_iH_kO_l$ and $C_iH_kO_lN_m$. With a ratio of (12–33):1, they appeared to be the most significant in the formation of the Moskovskaya sausage aroma. Furthermore, we established that carboxylic acid esters were the main class of compounds identified in all the samples. Their mass fraction ranged from 76.61% to 81.60% of the total substances.

The data revealed no relationship between the oxidative processes and the degree of casing permeability. The correlation analysis identified the main chemical compounds that increase the intensity of cooked smoked sausages.

The practical significance of the study lies in creating a database of over 200 aromatic compounds.

Table 7. Elemental composition of esters identified in the gas phase of Moskovskaya sausage samples

Ci in the ester molecule	Chemical formula of identified esters	Total amount of esters with Ci in the gas phase of samples, % of total substances			
		Sample no. 1	Sample no. 2	Sample no. 3	Sample no. 4
1	2	3	4	5	1
C_6	$C_6H_{12}O_2$	0.34	–	–	0.1
C_7	$C_7H_{14}O_2$	–	0.52	–	–
C_8	$C_8H_{16}O_4$	–	–	0.28	–
C_{10}	$C_{10}H_{18}O_2$, $C_{10}H_{20}O_2$	–	0.71	1.17	–
C_{11}	$C_{11}H_{19}ClO$, $C_{11}H_{21}ClO$, $C_{11}H_{22}O$	4.85	–	3.26	0.4
C_{12}	$C_{12}H_{20}O_2$, $C_{12}H_{24}O_2$	0.16	0.18	–	0.08
C_{13}	$C_{13}H_{15}N_3O_4$, $C_{13}H_{24}O_2$, $C_{13}H_{26}O_2$	–	0.38	–	0.32
C_{14}	$C_{14}H_{10}O_6$, $C_{14}H_{12}ClNO_3$, $C_{14}H_{28}O_2$	0.09	–	12.45	–
C_{15}	$C_{15}H_{30}O$, $C_{15}H_{30}O_2$	1.00	–	–	0.59
C_{16}	$C_{16}H_{11}F_3N_2O_2S$, $C_{16}H_{27}NO_3$	0.10	–	–	0.19
C_{17}	$C_{17}H_{32}O_2$, $C_{17}H_{34}O_2$	12.91	10.87	–	13.03
C_{19}	$C_{19}H_{36}O_2$, $C_{19}H_{36}O_3$, $C_{19}H_{36}O_4$, $C_{19}H_{38}O_2$, $C_{19}H_{38}O_4$	57.91	63.45	59.58	63.75
C_{20}	$C_{20}H_{28}O_6$, $C_{20}H_{40}O_2$	0.42	–	0.17	–
C_{21}	$C_{21}H_{32}O_2$, $C_{21}H_{42}O_2$	–	–	0.25	0.44
C_{22}	$C_{22}H_{18}F_6N_4O_4$, $C_{22}H_{44}O_2$	0.12	–	0.34	–
C_{23}	$C_{23}H_{32}N_2O_4$, $C_{23}H_{46}O_4$	–	0.5	–	2.7

Table 8. Correlation coefficients between aroma intensity ('electronic nose') and groups of substances in the samples gas phase

Groups of substances in the product gas phase	Correlation coefficient between groups of substances and aroma intensity
1	2
Substances with the general formula $C_iH_kO_l$	–0.9932
Substances with the general formula $C_iH_kO_lN_m$	–0.2812
All oxygen-containing substances	–0.9540
incl. oxygen-containing heterocycles	–0.5121
All nitrogen-containing substances	0.5812
incl. nitrogen-containing heterocycles (except amines, amides, nitriles, and hydrazides)	0.7927
All chlorine-containing substances	0.8128
Alcohols	–0.5419
Esters	–0.4805
incl. esters with total carbons C19	–0.7561

This database allows for a deeper understanding of aroma formation processes in cooked smoked sausages under various technological conditions. As a result, we can exert a purposeful influence on the quality indicators and create various flavour compositions to adjust the sensory properties of the finished product.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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