

Flavor Deterioration of Nonfermented Coarse-Cut Sausage during Storage. Flavor as a Factor of Quality for Nonfermented Sausage. 2

Mitsuya Shimoda,*† Hideki Shiratsuchi,† Yutaka Minegishi,‡ and Yutaka Osajima†

Department of Food Science and Technology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka-shi 812, Japan, and Central Research Institute, Marudai Food Company, Ltd., 21-3 Midori-cho, Takatsuki-shi, Osaka-fu 569, Japan

Flavor deterioration of a nonfermented sausage during storage was investigated. The flavor of sausage stored at 10 °C was evaluated periodically by trained panelists. It was found that the decrease in spice and smoke odors and the increase in stale and fatty odors caused flavor deterioration. Homogenate of the sausage and water containing 2-heptanol as an internal standard was distilled under reduced pressure and the distillate passed through a column packed with Porapak Q. Adsorbed volatiles were eluted with diethyl ether. The odor concentrate was analyzed by gas chromatography and gas chromatography-mass spectrometry. To determine the compounds contributing to flavor deterioration, the concentrate was fractionated by silica gel TLC and a preparative GC. 1,6-Dioxacyclododecane-7,12-dione, which increased about 4 times during storage, was responsible for a heavy and fatty odor. 3,4-Dimethyl-2(5*H*)-furanone, 2-hydroxy-3-methyl-2-cyclopenten-1-one, and δ -decanolactone increased by 2-3 times and produced a caramel-like sweet odor. The decreases in spicy and smoky odor were contrary to the increase in guaiacol and its derivatives and many terpenes during storage.

INTRODUCTION

Off-flavors develop when nonfermented sausage is stored for more than 20 days at 10 °C. Many studies have been conducted on the flavor of dry-cured hams or sausages (Berdague et al., 1991; Berger et al., 1990; Baloga et al., 1990), but there have been few studies on nonfermented sausage except for our previous paper (Shiratsuchi et al., 1993). For meats and their products, a review by Reineccius (1979) covered off-flavors due to genetics, animal diet, environment, processing, bacterial spoilage, and oxidation of lipids but provided little information on flavor deterioration during storage. To date, there have been a few studies on the flavor changes after processing of ham and sausage. For example, the contributions of the curing agents, sodium chloride and nitrite, to cured meat flavor have been investigated organoleptically (MacDonald et al., 1980a-c). The effect of lipolysis on carbonyl formation during ripening of a dry sausage was demonstrated to relate the change in flavor to the hydrolytic and oxidative conversions in a lipid fraction (Demeyer et al., 1974).

The aim of this study is to relate the flavor deterioration to the change in volatile compounds of a nonfermented sausage during storage.

MATERIALS AND METHODS

Materials. Nonfermented coarse-cut sausage was available from Marudai Food Co., Ltd., with these declared ingredients: lean pork (60%), steer meat (5%), pork fat (15%), salt (1%), sodium ascorbate (0.04%), NaNO₂ (0.02%), water (12%), and other ingredients (6.94%). The sausages, which were flavored by adding a mixed spice and spraying with a liquid smoke, had a potential shelf life of 25 days at 10 °C. In the present experiment, to evaluate flavor deterioration during storage, 300 g of the sausage was packed in a pouch of high gas barrier film and stored for 60 days at 10 °C. For chemical analysis of volatile compounds, the sausage was stored at 20 °C to deteriorate the flavor within a short period. Silica gel 60 F-254 plates for thin-

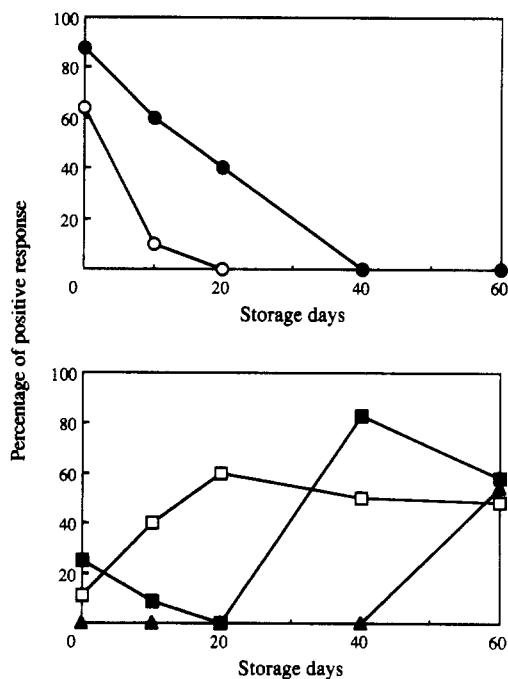


Figure 1. Sensory evaluation of nonfermented coarse-cut sausage flavor during storage at 10 °C. Sensory evaluation: Spicy, smoky, stale, fatty, and pungent odors were evaluated by 8-10 trained panelists on the dipole scale of "appreciable" and "not appreciable". (○) Spicy; (●) smoky; (□) stale; (■) fatty; (▲) pungent.

layer chromatography (TLC) were from Merck, and porous polymer beads, Porapak type Q, from Waters. High-purity solvents were from Wako Chemicals and Nakalai Tesque.

Sensory Test. The flavor of sausage stored at 10 °C was evaluated periodically by 8-10 trained panelists of the Marudai Food Co., Ltd. The flavor attributes spicy, smoky, fatty, stale, and pungent were evaluated on a dipole scale of 1 (appreciable) and 0 (not appreciable).

Isolation of Volatiles. The sausage (600 g) was homogenized in 900 mL of deionized water containing 0.6 mg of 2-heptanol as an internal standard. Volatiles were separated from the homogenate by a combination of steam distillation under reduced pressure (about 53 mmHg) and a column concentration. The

* Author to whom correspondence should be addressed.

† Kyushu University.

‡ Marudai Food Co., Ltd.

Table I. Change in Volatile Compounds from Spice in Nonfermented Sausage during Storage at 20 °C

peak	compound	Kovats index ^a	content, ^b ppb			
			0 days	5 days	10 days	20 days
3	3-methylbutanal	914	5	5	7	9
4	methyl isobutyrate	924	9	11	12	9
5	ethanol	930	17	81	72	60
6	α -pinene	1019	275	235	277	242
7	unknown	1022	60	55	60	50
11	β -pinene	1108	514	514	528	504
12	α -thujene	1120	777	811	804	783
16	Δ_3 -carene	1146	350	326	361	347
17	myrcene	1160	99	132	138	117
18	α -terpinene	1179	16	54	49	54
20	<i>d</i> -limonene	1198	845	888	920	824
21	β -terpinene	1206	44	74	76	76
22	1,8-cineole	1209	350	377	400	388
24	γ -terpinene	1243	192	219	222	224
25	1,2,3-trimethylbenzene	1257	12	14	14	13
26	<i>p</i> -cymene	1268	242	268	277	244
27	<i>trans</i> -ocimene	1276	22	25	26	22
29	terpinolene	1282	133	154	161	162
44	δ -elemene	1465	65	81	106	117
47	α -copaene	1487	51	79	110	113
57	linalool	1546	1161	1207	1379	1505
58	linalyl acetate	1553	48	57	66	88
59	sesquiterpene	1562	331	296	293	278
65	β -caryophyllene	1587	159	415	738	1341
66	4-terpineol	1602	446	541	613	564
69	2-furanmethanol	1659	475	541	606	523
70	α -caryophyllene	1667	56	86	123	127
73	α -terpineol	1697	171	179	223	279
76	methylphosphonic acid dimethyl ester	1718	40	44	56	58
81	unknown	1773	tr	tr	tr	tr
82	4-(1-methylethyl)benzaldehyde	1781	940	993	1196	1236
85	sabinol	1810	43	46	33	67
88	unknown	1845	tr	tr	tr	tr
89	hexanoic acid and <i>p</i> -cymen-8-ol	1851	167	168	193	188
94	2,6-di- <i>tert</i> -butyl- <i>p</i> -cresol	1910	85	87	124	123
95	unknown	1923	31	36	44	46
100	unknown	1989	44	47	68	66
105	unknown	2087	105	109	123	140
108	2,6-dimethoxyphenol	2269	809	820	981	884

^a Modified Kovats indices calculated for DB-Wax capillary column. ^b Concentration in the sausage.

adsorptive column (2 cm i.d. \times 10 cm) was prepared by packing 15 mL of Porapak Q resin. Volatile compounds adsorbed were eluted with 60 mL of ethyl ether.

Preparation of 1,6-Dioxacyclododecane-7,12-dione. Adipic acid (1.46 g) and 0.9 g of 1,4-butanediol were dissolved in 1 L of benzene containing 0.5 g of *p*-toluenesulfonic acid and then refluxed at 82 °C for 12 h. The mixture was washed three times with 1% Na₂CO₃ aqueous solution and three times with water. The solvent was evaporated under reduced pressure. The lower phase was separated and distilled *in vacuo* at 80 °C. The distillate gave crystalline product (120 mg, mp 95.2 °C). Its mass spectrum was in accord with that published (*The Wiley/NBS Registry of Mass Spectral Data*, 1989).

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). A Hewlett-Packard Model 5890 gas chromatograph equipped with a 60 m \times 0.25 mm i.d. DB-Wax, film thickness 0.25 μ m, fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) was used. The oven temperature was held at 50 °C for 4 min and then programmed to 230 °C at 2 °C/min. Helium carrier gas flow rate was 28 cm/s. A Shimadzu GCMS-9020DF gas chromatograph-mass spectrometer system was used for identification of the GC components. The column and oven conditions for GC-MS were as described for GC analysis.

Fractionation of Odor Concentrate. The odor concentrate from 12 kg of the sausage before and after storage (20 °C, 30 days) was fractionated by silica gel TLC (solvent, diethyl ether/isopentane 20:80). The polar fraction with fatty and sweet odors, which seemed to contribute directly to flavor deterioration, was further fractionated by preparative GC. For preparative GC, a Shimadzu GC 8A was used, equipped with a 40 m \times 1.2 mm i.d. chemically bonded PEG 20M (film thickness, 1 μ m) megabore

open tubular column (Chemicals Inspection and Testing Institute, Tokyo) and an FID. The oven temperature was programmed from 50 to 220 °C at 2 °C/min. Helium carrier gas flow rate was 18 mL/min with a splitless injection. Short capillaries (25 cm \times 0.53 mm i.d.) with a chemically bonded phase of PEG 20M were used for trapping separated fractions or components with a split ratio of 20:1. The separation port was especially designed so that a large negative temperature gradient could be produced in the middle of the capillary, and the capillary could be exchanged in a very short time. The separated compounds were eluted on a filter paper with a drop of ethyl ether, and the odor was sniffed.

RESULTS AND DISCUSSION

Figure 1 shows the changes in odor of sausage during storage at 10 °C. The ordinate indicates a percentage of the positive response. Spicy odor decreased rapidly during the early period of storage and was not perceived after 20 days. The smoke odor decreased more slowly and was not noted at 40 days. On the other hand, stale odor had increased heavily during the first 20 days, and a fatty odor developed rapidly after 20 days. A pungent odor began to develop after 40 days. It was considered that the decrease in spice and smoke odor and the increase in stale and fatty odors caused a flavor deterioration, but it was not considered that the pungent odor was responsible for the flavor deterioration, since the odor began to be perceived far beyond the shelf life of the sausage.

Because the flavors of spice, smoke, and meat constitute the principal flavor attributes of sausage, the volatile compounds derived from spice, liquid smoke, and meat

Table II. Change in Volatile Compounds from Smoke Liquid in Nonfermented Sausage during Storage at 20 °C

peak	compound	Kovats index ^a	content, ^b ppb			
			0 days	5 days	10 days	20 days
2	ethyl acetate	885	35	39	43	57
3	3-methylbutanal	914	5	5	7	9
4	methyl isobutyrate	924	9	11	12	9
5	ethanol	930	17	81	72	60
8	toluene	1035	416	515	632	521
13	ethylbenzene and (<i>E</i>)-2-pentenal	1125	10	8	9	11
19	<i>o</i> -xylene	1181	53	56	60	53
23	1-ethyl-3-methylbenzene	1222	20	25	18	23
27	<i>trans</i> -ocimene	1276	22	25	26	22
28	α -phellandrene	1279	29	21	23	21
29	terpinolene	1282	133	154	161	162
30	tridecane	1300	23	17	17	17
32	1-hexanol	1353	102	83	104	95
33	2-methyl-2-cyclopenten-1-one	1367	125	148	139	126
37	unknown	1424	45	37	44	36
40	unknown	1439	7	16	32	51
41	unknown	1442	49	40	42	34
43	2-furfural	1457	1106	884	739	468
45	diallyl disulfide	1475	55	53	60	52
49	1-(2-furanyl)ethanone	1497	43	28	40	48
51	camphor	1511	55	55	63	53
53	3-oxopentanoic acid methyl ester	1522	88	81	76	51
54	1-(acetyloxy)-2-butanone	1526	95	85	78	67
55	unknown	1531	27	36	47	41
56	2,3-dimethyl-2-cyclopenten-1-one	1535	115	125	138	144
59	sesquiterpene	1562	331	296	293	278
60	isopulegol	1567	42	61	60	52
67	butanoic acid	1630	103	101	115	103
68	3,5,5-trimethyl-2-cyclopenten-1-one	1644	118	124	134	142
69	2-furanmethanol	1659	475	541	606	523
70	α -caryophyllene	1667	56	86	123	127
71	3,3-dimethylbicyclo[2.2.1]heptan-2-one	1680	91	100	110	108
75	3-methyl-2(5 <i>H</i>)-furanone	1713	74	96	115	118
78	unknown	1726	17	20	27	23
81	unknown	1773	tr	tr	tr	tr
84	2,4-dimethyl-1,3-cyclopentanedione	1796	88	82	107	113
86	2-hydroxy-3-methyl-2-cyclopenten-1-one	1830	573	595	654	631
90	guaiacol	1859	1429	1476	1660	1828
91	unknown	1868	41	43	54	56
92	5-(2-propenyl)-1,3-benzodioxole	1873	64	68	79	88
93	2,4-dihydro-2,4,5-trimethyl-3 <i>H</i> -pyrazol-3-one	1896	158	164	186	193
94	2,6-di- <i>tert</i> -butyl- <i>p</i> -cresol (BHT)	1910	85	87	124	123
97	4-methylguaiacol	1956	777	810	987	1057
98	unknown	1968	68	74	85	78
99	unknown	1977	44	47	58	55
100	unknown	1989	44	47	68	66
101	phenyl carbamate	2006	353	356	407	425
102	4-ethylguaiacol	2032	148	161	204	220
104	4-methylphenol	2079	144	149	181	196
105	unknown	2087	105	109	123	140
108	2,6-dimethoxyphenol	2269	809	820	981	884
109	eugenol	2281	17	22	19	20
111	1,2,3-trimethoxybenzene	2335	333	365	425	450
112	1-(2,6-dihydroxy-4-methoxyphenyl)ethanone	2413	455	342	276	235
113	1,4-benzenediol		962	1487	1074	1014

^{a,b} Same as in Table I.

should be separately considered. In a previous paper (Shiratsuchi et al., 1993), we reported the identification, quantification, and source materials of volatile compounds in sausage. To correlate the change in sausage flavor with the quantitative change of volatile compounds, the volatile compounds in sausage were determined periodically. The quantitative values of volatile compounds derived from the mixed spice are listed in Table I. Several components such as α - and β -terpinene, δ -elemene, α -copaene, linalool, linalyl acetate, β -caryophyllene, α -caryophyllene, and α -terpineol increased during storage, while other components rarely changed in their quantities. As shown in our previous paper (Shiratsuchi et al., 1993), some terpenes increased with processing, and this paper showed that these compounds continued to increase also during storage. β -Caryophyllene especially increased more than 8 times

during 20 days at 20 °C. None of the compounds included in Table I decreased significantly. As a result, the decrease in spicy odor during storage could not be correlated with change in the quantitative values of compounds listed in Table I. In the future, the contributions of trace and sensory important components to spice odor should be elucidated. Table II lists the quantitative values of volatile compounds derived from liquid smoke. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, and 1,2,3-trimethoxybenzene significantly increased, while 2-furfural decreased greatly. The increase in guaiacol and its derivatives with a typical smoke odor was contrary to the decrease in smoke odor during storage. The synergism of odorous compounds to the sense of smell should also be taken into account, and we are further concerned with the investigation of volatile compounds in liquid smoke. Table III lists the

Table III. Change in Volatile Compounds in Nonfermented Sausage without the Compounds from Spice and Smoke Liquid during Storage at 20 °C

peak	compound	Kovats index ^a	content, ^b ppb			
			0 days	5 days	10 days	20 days
1	unknown	821	10	12	16	21
9	hexanal	1079	28	29	29	23
10	2-methylthiophene and 2-methyl-2-butenal	1093	10	19	25	27
14	<i>p</i> -xylene	1132	12	13	13	10
15	<i>m</i> -xylene	1138	30	30	30	26
31	ethyl lactate	1341	26	33	57	80
34	unknown	1374	147	168	178	178
35	6-ethyl-2-methyldecane	1390	26	28	29	32
36	unknown	1396	21	23	26	28
38	ethyl octanoate	1429	32	25	25	20
39	unknown	1432	40	41	39	39
42	(<i>E,E</i>)-2,4-hexadienoic acid methyl ester	1444	64	66	73	88
46	benzenethiol	1480	tr	tr	tr	tr
48	unknown	1493	164	163	167	155
50	(<i>E,E</i>)-2,4-hexadienoic acid ethyl ester	1501	15	20	18	17
52	benzaldehyde and 3-methyl-2-cyclopenten-1-one	1513	141	151	160	162
61	γ -caryophyllene	1573	212	419	637	305
62	unknown	1577	80	54	60	71
63	3-methoxypyridine	1579	tr	9	16	16
64	β -elemene	1583	tr	10	22	24
72	neoisothujyl alcohol	1694	25	27	35	37
74	unknown	1704	tr	tr	tr	tr
77	4,5-dimethyl-4-hexen-3-one	1722	61	68	80	84
79	unknown	1757	30	36	44	54
80	unknown	1767	69	61	70	57
83	3,4-dimethyl-2(5 <i>H</i>)-furanone	1789	23	33	36	41
87	unknown	1838	tr	tr	tr	tr
96	4-methoxy-1,3-benzenediamine	1942	32	38	38	43
103	octanoic acid	2053	72	82	120	160
106	(<i>E,E</i>)-2,4-hexadienoic acid	2150	4808	5310	6322	5724
107	δ -decanolactone		12	15	17	29
110	1,6-dioxacyclododecane-7,12-dione		6	11	16	23

^{a,b} Same as in Table I.

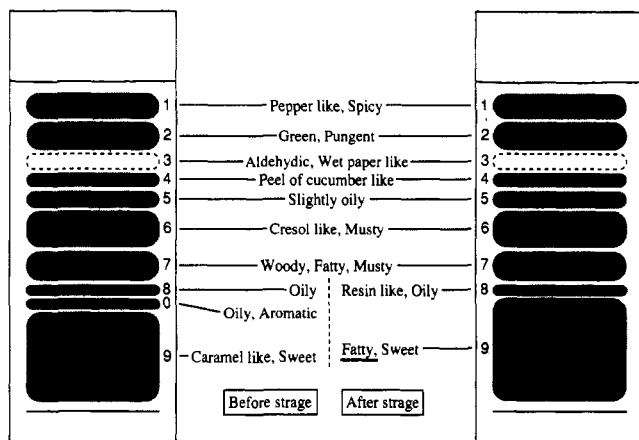


Figure 2. Thin-layer chromatograms of odor concentrate of nonfermented sausage before and after storage. The sausage was stored for 30 days at 20 °C.

quantitative values of volatile compounds not found in the mixed spice and liquid smoke. Terpenes could be derived from other terpenes contained in the spice and liquid smoke. The increase of terpenes during storage supports this suggestion. 2-Methylthiophene or 2-methyl-2-butenal increased, and it seemed to influence the change in the top note of sausage. 3,4-Dimethyl-2(5*H*)-furanone and δ -decanolactone, which increased almost twice after 20 days, invested a sweet flavor in the sausage, and this flavor attribute might suppress the flavors of spice and smoke. Octanoic acid might result from oxidative degradation of unsaturated fatty acid and ethyl lactate increased by esterification of lactate. These two compounds might serve as effective indices for the quality deterioration of sausages and hams during storage.

To clarify the compounds which directly contribute to flavor deterioration, the fractionation of odor concentrate was carried out. Thin-layer chromatograms of the odor concentrates before and after storage (30 days at 20 °C) are shown in Figure 2. Fraction 1, having a spicy or pepper-like odor, was well preserved even after storage, although the strength of the spicy odor in the sausage decreased rapidly as shown in Figure 1. Fractions 6 and 7, having a smoky odor, also scarcely decreased, while its odor with the sausage decreased. These contrary findings were consistent with the results of Tables I and II. The odor of fraction 9 changed from a sweet and caramel-like odor to a sweet and fatty odor, and the latter was similar to the odor attribute of off-flavor. Therefore, it was suggested that off-flavor compounds could be included in fraction 9. Then the eluates of fraction 9 before and after storage were applied to preparative GC. As shown in Figure 3, fraction 9 before and after storage was separated into nine portions. This figure showed that odors of fractions D, F, and G greatly changed during storage. That is, the odor of fraction D changed from aromatic to caramel odor, fraction F from aromatic and oily to ham-like and cresol-like odors, and fraction G from spicy and pungent to fatty and sour odors. It was considered that the off-flavor compounds were included in these three fractions (D, F, and G). Every peak of the three fractions, therefore, was separated to sniff its odor with preparative GC. Peak 1, 3,4-dimethyl-2(5*H*)-furanone, had a peanut-butter-like odor; peak 2, 2-hydroxy-3-methyl-2-cyclopenten-1-one, a sweet and caramel-like odor; peak 3, hexanoic acid, a pungent and sour odor; peak 4, δ -decanolactone, a coconut-like odor; and peak 5, 1,6-dioxacyclododecane-7,12-dione, a heavy fatty odor. These compounds were considered to be the compounds contributing to the flavor deterioration

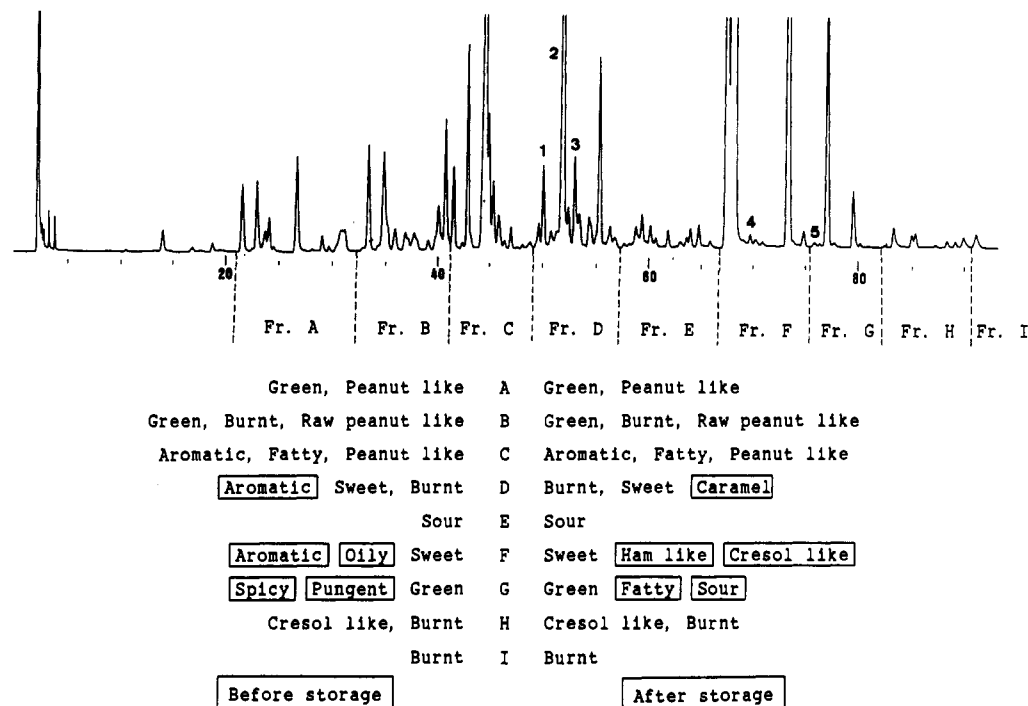


Figure 3. Sniffing of the fraction obtained by preparative gas chromatography on fraction 9 of the thin-layer chromatogram. For more information, see Materials and Methods.

of nonfermented coarse-cut sausage, since they had strong odors associated with the off-flavor which increased during storage (Tables I-III).

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Registry No. Supplied by Author: 3-Methylbutanal, 590-86-3; methyl isobutyrate, 547-63-7; ethanol, 64-17-5; α -pinene, 7785-70-8; β -pinene, 18172-67-3; α -thujene, 3387-41-5; carene, 13466-78-9; myrcene, 123-35-3; α -terpinene, 99-85-6; *d*-limonene, 5989-27-5; β -terpinene, 99-84-3; 1,8-cineole, 470-82-6; γ -terpinene, 99-85-4; 1,2,3-trimethylbenzene, 526-73-8; *p*-cymene, 99-81-6; *trans*-ocimene, 3338-

55-4; terpinolene, 586-62-9; δ -elemene, 20307-84-0; α -copaene, 3856-25-5; linalool, 78-70-6; linalyl acetate, 115-95-7; β -caryophyllene, 13877-93-5; 4-terpineol, 562-74-3; 2-furanmethanol, 98-00-0; α -caryophyllene, 6753-98-6; α -terpineol, 98-55-5; methylphosphonic acid dimethyl ester, 756-79-6; 4-(1-methylethyl)benzaldehyde, 122-03-2; sabinol, 471-16-9; hexanoic acid, 142-62-1; *p*-cymen-8-ol, 99-87-6; 2,6-di-*tert*-butyl-*p*-cresol, 128-37-0; 2,6-dimethoxyphenol, 91-10-1; ethylacetate, 141-78-6; toluene, 108-88-3; ethylbenzene, 100-41-4; (*E*)-2-pentenal, 1576-87-0; *o*-xylene, 95-47-6; 1-ethyl-3-methylbenzene, 620-14-4; α -phellandrene, 99-83-2; tridecane, 629-50-5; 1-hexanol, 111-27-3; 2-methyl-2-cyclopenten-1-one, 1120-73-6; 2-furfural, 98-01-1; diallyl disulfide, 2179-57-9; 1-(2-furanyl)ethanone, 1192-62-7; camphor, 76-22-2; 3-oxopentanoic acid methyl ester, 2067-33-6; 1-(acetyloxy)-2-butanone, 1575-57-1; 2,3-dimethyl-2-cyclopenten-1-one, 1121-05-7; 3,3-dimethylbicyclo[2.2.1]heptan-2-one, 13211-15-9; 3-methyl-2(5*H*)-furanone, 22122-36-7; 2,4-dimethyl-1,3-cyclopentanedione, 34598-80-6; 2-hydroxy-3-methyl-2-cyclopenten-1-one, 765-69-5; guaiacol, 90-05-1; 5-(2-propenyl)-1,3-benzodioxole, 94-59-7; 2,4-dihydro-2,4,5-trimethyl-3*H*-pyrazol-3-one, 17826-82-3; 4-methylguaiacol, 93-51-6; 4-ethylguaiacol, 2785-89-9; 4-methylphenol, 106-44-5; 2,6-dimethoxyphenol, 91-10-1; eugenol, 97-53-0; 1,2,3-trimethoxybenzene, 634-36-3; 1-(2,6-dihydroxy-4-methoxyphenyl)ethanone, 7507-89-3; 1,4-benzenediol, 123-31-9; 1-hexanal, 66-25-1; 2-methylthiophene, 554-14-3; 2-methyl-2-butenal, 497-03-0; *p*-xylene, 106-42-3; *m*-xylene, 108-38-3; ethyl lactate, 687-47-8; 6-ethyl-2-methyldecane, 62108-21-8; ethyl octanoate, 106-32-1; (*E,E*)-2,4-hexadienoic acid methyl ester, 1515-80-6; benzenethiol, 108-98-5; (*E,E*)-2,4-hexadienoic acid ethyl ester, 5941-48-0; benzaldehyde, 100-52-7; γ -caryophyllene, 118-65-0; 3-methoxypyridine; β -elemene, 515-13-9; neoisothujyl alcohol, 31187-53-8; 4,5-dimethyl-4-hexen-3-one, 17325-90-5; 3,4-dimethyl-2(5*H*)-furanone, 1575-46-8; 4-methoxy-1,3-benzenediamine, 615-05-4; octanoic acid, 124-07-2; (*E,E*)-2,4-hexadienoic acid, 110-44-1; δ -decanolactone, 705-86-2; 1,6-dioxacyclododecane-7,12-dione, 777-95-7.