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Volatile Constituents of Dry Elder (*Sambucus nigra* L.) Flowers

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Three extracts of dry elder flowers, *Sambucus nigra* L., were analyzed: a steam-distilled essential oil, an ethanol concentrate of a petroleum ether extraction, and an isopentane extract of an ethanol concentrate. By use of gas chromatography, infrared spectroscopy and mass spectrometry, 79 compounds were identified: 16 hydrocarbons, 11 ethers and oxides, 7 ketones, 7 aldehydes, 16 alcohols, 6 esters, and 16 acids. The major constituents of the essential oil were *trans*-3,7-dimethyl-1,3,7-octatrien-3-ol (13%), palmitic acid (11.3%), linalool (3.7%), *cis*-hexenol (2.5%), and *cis*- and *trans*-rose oxides (3.4 and 1.7%, respectively). They were also principal components of the isopentane extract and of the ethanol concentrate. The three extracts had a good muscat odor.

Elder, *Sambucus nigra* L., is a wild shrub, which may grow to 10 m. Its white flowers possess a pleasant strong smell. A high-priced wine is produced in England from the berries. To our knowledge, the plant is not cultivated. Flowers are harvested around May–June and dried away from sunlight at a temperature lower than 40 °C, to minimize aroma loss.

Steiner and Von Kamiensky (1953) reported ethylamine, isobutylamine, and isoamylamine in elder flowers. Leifertova and Kudrnacova (1971) reported high levels of phenolic compounds in the buds and found choline in the flowers but not in the berries. Willuhn (1974) identified several alkanes in the elder leaves: heptacosane, nonacosane, and hentriacontane being quantitatively the most important ones. Richter and Willuhn (1974) reported that the essential oil of elder flowers was high in fatty acids (66%) and *n*-alkanes (7.2%). According to Bonnier and de Layens (1970), apples stored on a dry elder flowers bed have a pleasant muscat like aroma. This was in agreement with Bayonove (1973), who imitated muscat wine by adding dry elder flowers to a Grenache wine and reported that a sensory panel did not identify the true muscat wine. These facts led to our interest in studying further the composition of elder flowers aroma.

EXPERIMENTAL SECTION

Materials. Three extracts were studied: a steam distilled oil, an ethanol concentrate, and an isopentane extract. From 400 g of dry elder flowers with a water content of 96 ± 0.5 g/kg of dry matter and provided by Ducros Co. (Buis-les-Baronnies, France), an essential oil was steam distilled for 24 h on a modified Clevenger apparatus (Miquel et al., 1976). To avoid any loss of oil, 2 mL of ethyl ether–pentane (1:1) were introduced in the separator. Solvents were evaporated under a nitrogen stream and essential oil was kept at −18 °C. Its acid index was 70 ± 3.5 mg of KOH/g of essential oil. The yield was 0.53 g/kg of dry elder flowers. From a distilled ethyl alcohol extract obtained after soaking 150 g of the same sample of elder flowers in 1 L of alcohol (45%) at room temperature, a volatile essence was recovered by liquid–liquid extraction using purified isopentane. The yield of this isopentane extract was 0.15 g/kg of dry elder flowers. From an oleoresin prepared by soaking under reflux dry elder flowers in petroleum ether, an ethanol concentrate was provided by Payan-Bertrand (Grasse, France).

Purification of the Essential Oil and Identification of Its Constituents. *Separation by Column Chromatography.* A glass column, 30 cm × 2 cm i.d., filled with Florisil 60–100-mesh ASTM to the 20-cm level, was prewashed with 100 mL of distilled anhydrous petroleum ether. Elder flowers essential oil (1 g) was poured at the top of the column and eluted successively with petroleum ether, petroleum ether–ethyl ether (9:1), petroleum ether–ethyl ether (1:1), ethyl ether, and finally methanol. Solvents were removed under vacuum in a rotary evaporator and five final fractions were obtained.

Separation by Gas–Liquid Chromatography. A thermal conductivity detector was used with a helium flow rate of 70 mL/min. The column was programmed from 60 to 220 °C at 6 °C/min and held.

Further purification was achieved either on the same instrument with another 4 m × 4 mm i.d. glass column

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packed with 5% SE-30 on 60–80-mesh AW-DMCS Chromosorb G or on a Varian 1420 equipped with a stainless steel $^1/_8$ in. packed with 5% Carbowax 20 M on 80–100–mesh AW-DMCS Chromosorb G. The Varian GLC had a helium flow rate of 25 mL/min, and the column was programmed from 60 to 200 °C at 4 °C/min and held. Compounds were trapped in thin-walled glass capillaries according to the procedure described by Jennings (1970).

Identification of Volatiles. Identification was based on GLC retention data, infrared (IR), and mass spectrometry. IR spectra of purified liquid compounds were taken from thin films between sodium chloride plates on a Perkin-Elmer Model 297. IR spectra of purified solid compounds were taken in pressed KBr pellets. Gas chromatography–mass spectrometry was used to identify trace compounds present in the elder flowers essential oil. Analyses were run either on a Girdel 3000 chromatograph equipped with a $^1/_8$-in. column packed with Carbowax 20 M and connected to a Varian MAT CH 5 or on a Finnigan 9500 chromatograph equipped with a glass capillary column (42 m × 0.20 mm i.d.), FFAP coated and interfaced to a quadrupole Finnigan 3100. Ionization energy was 70 eV in both cases.

Quantitative Analysis of the Three Extracts. Quantitative analysis of the three different extracts utilized a 60 m × 0.4 mm i.d. glass capillary column, made in the laboratory on a Hupe Model 7501 glass capillary drawing machine, treated with HCl according to Alexander and Rutten (1973), and coated with FFAP following the static procedure described by Bouche and Verzele (1968). The column was connected to a Girdel 300 gas chromatograph equipped with a Chrompack splitter.

A 0.1-μL aliquot of essential oil, isopentane extract, or ethanol concentrate was injected with a split ratio of 1:10. Nitrogen carrier gas flow rate in the column was 3 mL/min, and column temperature was programmed from 60 to 210 °C at 1 °C/min and held.

The chromatograph was connected to a digital integrator, Varian Model CDS 111 C. Response factors were not used in calculating percentage composition from the integrator data.

Fatty Acid Content of the Three Extracts. A 200-mg portion of each of the three extracts (steam-distilled essential oil, isopentane extract, and ethanol concentrate) was neutralized with 0.1 N NaOH and then washed with petroleum ether. Salt solution was acidified to pH 1 with 0.1 N HCl to recover fatty acids which were converted to methyl esters according to the method of Christopherson and Glass (1969), modified by Prevot and Mordret (1976).

RESULTS AND DISCUSSION

The compounds purified and identified from the essential oil are reported in Table I.

Chromatograms of methyl esters (Figure 1) show several acids: tridecanoic, myristic, pentadecanoic, palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic.

The fatty acid percentage composition (Table II) of the three different extracts was calculated from the methyl ester chromatograms. It indicates large variations: palmitic acid was predominant in the essential oil (37.8%), whereas linoleic and palmitic acids had approximately the same level in the ethanol concentrate and in the isopentane extract.

Quantitative analysis of the three extracts are reported on Figure 2 and Table I. Differences among the three extracts clearly indicate that none of the extraction procedures yields an extract typical of the elder flower's aroma. However, the ethanol concentrate or isopentane

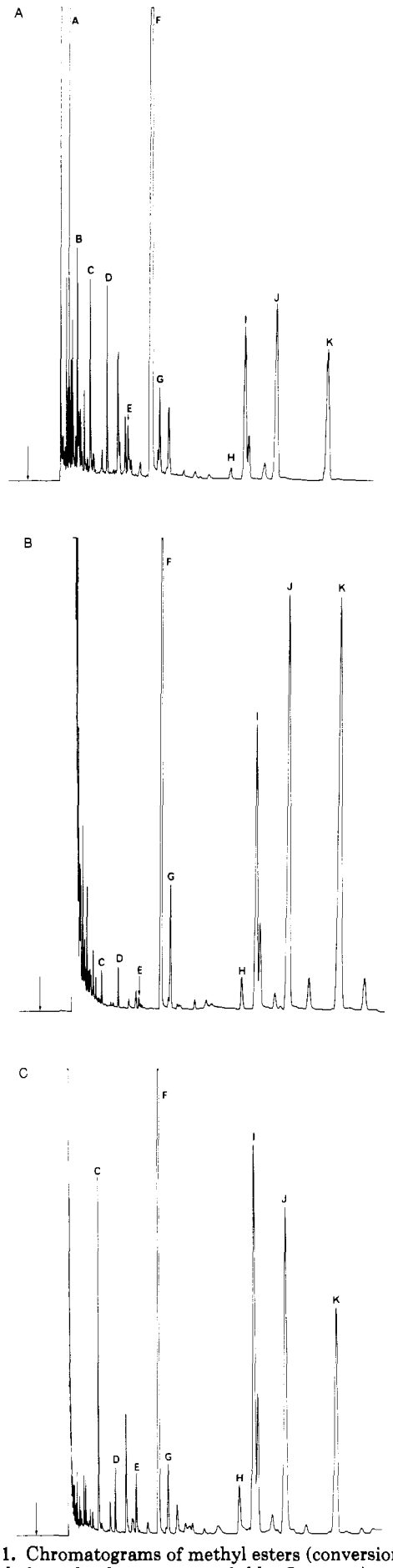

Figure 1. Chromatograms of methyl esters (conversions of the fatty acids from the three extracts of elder flowers: A = essential oil; B = isopentane extract; C = ethanol concentrate).

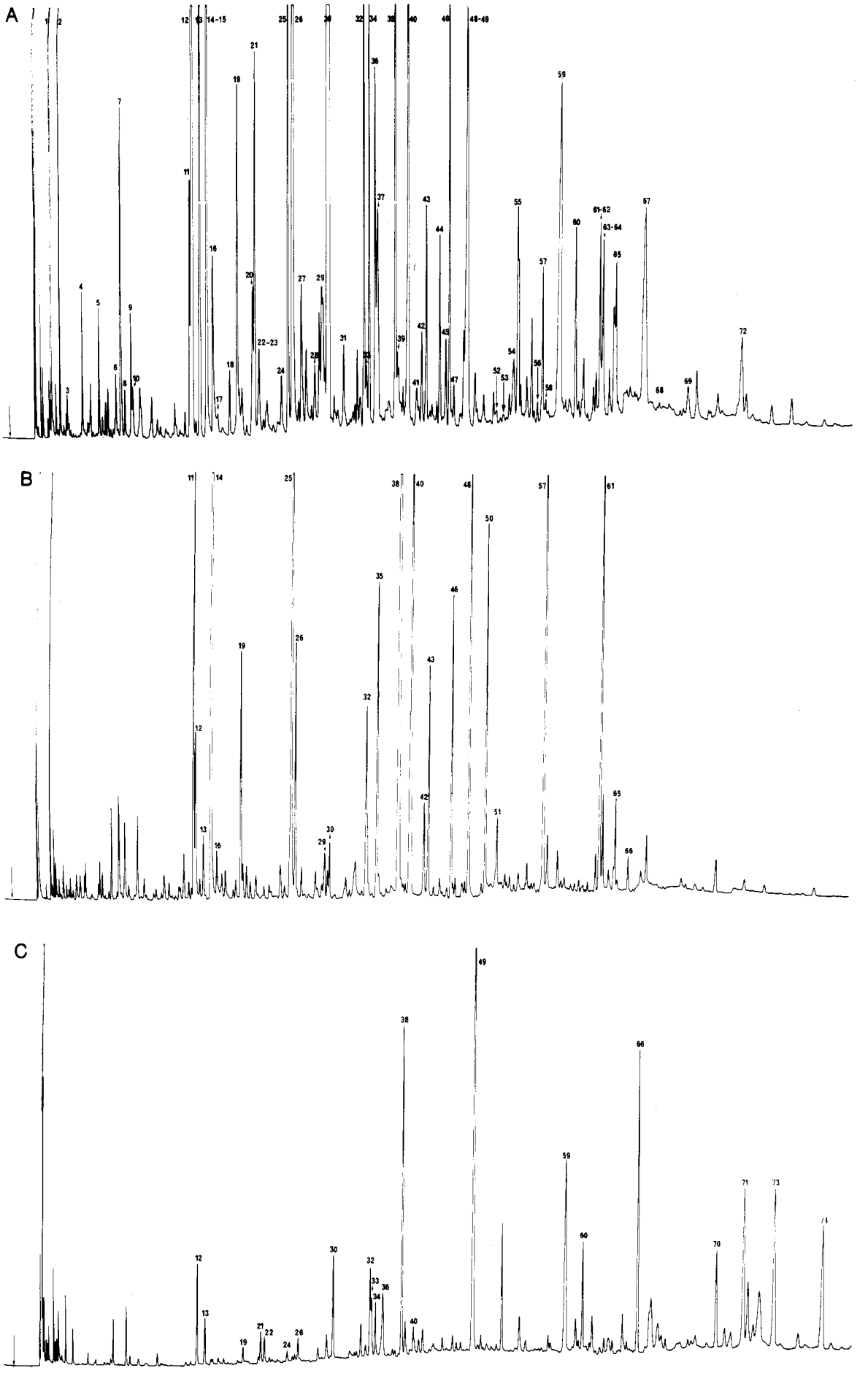

Figure 2. Chromatograms of the three extracts of elder flowers on a FFAP capillary glass column: A = essential oil; B = isopentane extract; C = ethanol concentrate.

Table I. Compounds Identified from the Essential Oil and Percentage Composition (Weight/Weight) of the Three Extracts of Elder Flowers

peak no.	compound	identification[a]	essential oil	isopentane extract	absolute essence
1	2-methylbutanal	MS	1.8		
2	unidentified		0.8		
3	toluene	MS	0.01		
4	hexanal	MS	0.2		
5	myrcene	MS	0.2		
6	heptanal	MS	0.1		
7	limonene	MS	0.7		
8	1,8-cineol	MS, IR	0.1		
9	*trans*-2-hexenal	MS, IR	0.3		
10	2-*n*-pentylfuran	MS	0.1		
11	*n*-hexanol	IR, EC	0.5	2.6	
12	*cis*-rose oxide	IR	3.4	1.2	1.2
13	*trans*-rose oxide	IR	1.7	0.4	0.6
14	*cis*-3-hexenol	MS, IR	2.5	10.1	
15	*n*-tetradecan	MS, EC			
16	nonanal	IR, EC	0.6	0.5	
17	α-thujone	MS, EC	0.07		
18	β-thujone	MS, EC	0.14		
19	linalool oxide A	MS, IR	0.9	1.7	0.2
20	linalool oxide B	MS, IR	0.4		
21	nerol oxide	MS, IR	0.9		0.4
22	*n*-pentadecan	MS, EC			
23	α-copaene	MS	0.3		0.4
24	camphor	MS, EC	0.2		0.3
25	benzaldehyde	MS, IR	1.2	6.3	
26	linalool	MS	3.7	1.7	0.4
27	unidentified		0.5		
28	*n*-hexadecane	MS, EC	0.2		
29	2-undecanone	MS	0.7	0.6	
30	Ho-trienol	MS, IR	13.7	0.6	1.4
31	menthol	MS, IR	0.3		
32	estragole	MS, IR	1.2	1.9	1.2
33	*n*-heptadecane	MS, EC	0.2		0.9
34	4-vinylanisole	IR	1.0		1.0
35	unidentified (close to Ho-trienol)			2.5	
36	α-terpineol	MS, IR	0.9		1.8
37	unidentified		0.8		
38	linalool oxide C/D	MS, IR	1.8	15.1	4.2
39	carvone	MS, IR	0.1		
40	citronellol	MS, IR	0.6	1.7	0.8
41	*n*-octadecane	MS, EC	0.1		
42	methyl salicylate	MS, IR	0.3	0.3	0.9
43	nerol	MS, IR	0.6	0.6	1.7
44	damascenone	MS, IR	0.6		
45	*trans*-anethole	MS, IR	0.3		
46	geraniol	IR, EC	1.0	2.0	
47	caproic acid	IR, EC	0.02		
48	benzylic alcohol	MS	2.1	4.0	
49	*n*-nonadecane	MS, EC			
50	phenylethanol	MS		2.6	
51	BHT (artifact)	MS		0.9	
52	β-ionone	MS	0.06		
53	oenanthic acid	EC	0.03		
54	*n*-eicosane	MS, EC, IR	0.4		
55	unidentified		0.9		
56	nerolidol	MS	0.07		
57	*p*-anisaldehyde	MS, IR	0.4	5.6	
58	caprylic acid	IR, EC	0.1		
59	*n*-heneicosane	MS, EC, IR	2.2		3.9
60	unidentified		0.6		1.4
61	eugenol	MS, IR	0.6	4.3	
62	pelargonic acid	IR, EC			
63	thymol	MS, IR	0.7		
64	*n*-docosane	MS, EC			
65	carvacrol	MS, EC	0.6	1.0	
66	ethyl palmitate	MS		0.8	4.0
67	*n*-tricosane	MS, EC, IR	1.7		
68	*trans*-geranic acid	IR	0.1		
69	*n*-tetracosane	MS, EC	0.2		
70	unidentified				1.9
71	unidentified				3.7
72	*n*-pentacosane	MS, EC, IR	0.6		
73	unidentified				3.8

Table I (*Continued*)

peak no.	compound	identification[a]	essential oil	isopentane extract	absolute essence
74	unidentified				3.3
A	undecanoic acid	EC	0.9		
B	lauric acid	EC	0.6		
C	tridecanoic acid	EC	0.7		0.4
D	myristic acid	EC	0.6		0.1
E	pentadecanoic acid	EC	0.2		0.1
F	palmitic acid	EC	11.3		2.0
G	palmitoleic acid	EC	0.5		0.2
H	stearic acid	EC	0.1		0.1
I	oleic acid	EC	1.7		1.8
J	linoleic acid	EC	2.7		1.9
K	linolenic acid	EC	2.8		1.6

[a] MS, mass spectra; IR, infrared spectra; EC, enriched chromatogram.

Table II. Fatty Acid Percentage Composition (Weight/Weight) of the Three Extracts (Calculated from the Methyl Esters Chromatograms)

peak no.	acid	essential oil	isopentane extract	ethanol concentrate
A	undecanoic	3		
B	lauric	2		
C	tridecanoic	2.4	0.5	3.9
D	myristic	2.1	0.4	1.0
E	pentadecanoic	0.8	0.2	1.2
F	palmitic	37.8	16.6	19.4
G	palmitoleic	1.5	3.2	1.8
H	stearic	0.4	0.7	0.7
I	oleic	5.7	7.9	18.0
J	linoleic	9.0	17.5	19.0
K	linolenic	9.1	24.0	16.0

extract was closer to the native aroma than the essential oil obtained by the more drastic procedure of hydrodistillation responsible for some artifacts and loss of polar volatiles.

The presence of BHT in the isopentane extract is due to the solvent.

Most of the results published by Richter and Willuhn (1974) were confirmed, except for the free fatty acids where we found half the amount reported in the essential oil by these authors. However, this large amount of free fatty acids is characteristic of elder flower's essential oil.

It is interesting to point out that isopentane extract does not possess any free fatty acids but contains ethyl palmitate which probably results from an esterification reaction of palmitic acid with ethanol during the extraction procedure. This kind of modification was already reported by De Smedt and Liddle (1976).

The drying process of elder flowers, away from sunlight at a temperature lower than 40 °C, but in the presence of air, thus of oxygen, allows ideal conditions for the oxidation of linoleic and linolenic acids probably with a lipoxygenase. So, this drying process is undoubtedly responsible of the presence of hexanal, heptanal, *trans*-2-hexenal, nonanal, and hexanol.

It should be emphasized that terpene alcohols represent at least 26% of the total essential oil, only 8% of the ethanol concentrate, and 30% of the isopentane extract. Among them, linalool (peak no. 26), *trans*-3,7-dimethyl-1,3,7-octatrien-3-ol (Ho-trienol, peak no. 30), α-terpineol (peak no. 36), nerol (peak no. 43), geraniol (peak no. 46), and linalool oxides (peaks no. 19 and 20) are considered as key compounds of muscat wine aroma (Cordonnier, 1956; Stevens et al., 1966; Bayonove et al., 1976).

Ho-trienol is present in a relatively large amount, 70 mg/kg of dry elder flowers. Its powerful smell, which resembles linden blossom aroma, is also similar to muscat. It is interesting to point out that Terrier (1972) called it muscat compound. Perhaps this similarity can be taken into account for the use of dry elder flowers to provide or reinforce muscat aroma.

Registry No. 2-Methylbutanal, 96-17-3; toluene, 108-88-3; hexanal, 66-25-1; myrcene, 123-35-3; heptanal, 111-71-7; limonene, 138-86-3; 1,8-cineol, 470-82-6; *trans*-2-hexenal, 6728-26-3; 2-*n*-pentylfuran, 3777-69-3; 1-hexanol, 111-27-3; *cis*-rose oxide, 3033-23-6; *trans*-rose oxide, 5258-11-7; *cis*-3-hexenol, 928-96-1; *n*-tetradecane, 629-59-4; nonanal, 124-19-6; α-thujone, 546-80-5; β-thujone, 471-15-8; linalool oxide A, 34995-77-2; linalool oxide B, 5989-33-3; nerol oxide, 1786-08-9; *n*-pentadecane, 629-62-9; α-copaene, 3856-25-5; camphor, 76-22-2; benzaldehyde, 100-52-7; linalool, 78-70-6; *n*-hexadecane, 544-76-3; 2-undecanone, 112-12-9; Ho-trienol, 20053-88-7; menthol, 1490-04-6; estragole, 140-67-0; *n*-heptadecane, 629-78-7; 4-vinylanisole, 637-69-4; α-terpineol, 98-55-5; linalool oxide C/D, 14049-11-7; carvone, 99-49-0; citronellol, 106-22-9; *n*-octadecane, 593-45-3; methyl salicylate, 119-36-8; nerol, 106-25-2; damascenone, 23726-93-4; *trans*-anethole, 4180-23-8; geraniol, 106-24-1; caproic acid, 142-62-1; benzylic alcohol, 100-51-6; *n*-nonadecane, 629-92-5; phenylethanol, 60-12-8; β-ionone, 79-77-6; oenanthic acid, 111-14-8; *n*-eicosane, 112-95-8; nerolidol, 7212-44-4; *p*-anisaldehyde, 123-11-5; caprylic acid, 124-07-2; *n*-heneicosane, 629-94-7; eugenol, 97-53-0; pelargonic acid, 112-05-0; thymol, 89-83-8; *n*-decosane, 629-97-0; carvacrol, 499-75-2; ethyl palmitate, 628-97-7; *n*-tricosane, 638-67-5; *trans*-geranic acid, 4698-08-2; *n*-tetracosane, 646-31-1; *n*-pentacosane, 629-99-2; undecanoic acid, 112-37-8; lauric acid, 143-07-7; tridecanoic acid, 638-53-9; myristic acid, 544-63-8; pentadecanoic acid, 1002-84-2; palmitic acid, 57-10-3; palmitoleic acid, 373-49-9; stearic acid, 57-11-4; oleic acid, 112-80-1; linoleic acid, 60-33-3; linolenic acid, 463-40-1.

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Lipid Deterioration Initiated by Phagocytic Cells in Muscle Foods: β-Carotene Destruction by a Myeloperoxidase-Hydrogen Peroxide-Halide System

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In fresh muscle foods, phagocytic cells may conceivably initiate and accelerate lipid oxidation. Fish leukocytes were obtained by density gradient centrifugation. The isolated neutrophils were rich in myeloperoxidase which was extracted from the leukocytes at pH 4.0 in the presence of 0.3 M sucrose. The crude enzyme showed a peroxidase activity of about 160 nmol of purpurogallin (formed from pyrogallol) (mg of protein)$^{-1}$ min^{-1}. An oxidation system using discoloration of β-carotene as an index of lipid peroxidation was developed. Myeloperoxidase from fish leukocytes caused rapid discoloration of β-carotene in the presence of H_2O_2 and iodide or bromide ions. Purified myeloperixidase cooxidized β-carotene in the presence of chloride ions. No destruction of β-carotene occurred when halogen ions, H_2O_2, or the enzyme was omitted from the system. The data indicate that leukocytes may be a focus for initiation of lipid peroxidation in biological tissues.

Oxidative degradation of lipids is a common and in some cases a major cause of deterioration of a wide range of foods, especially muscle foods, following harvesting or processing (Pearson et al., 1977). A critical question in the control of lipid oxidation concerns the source of the primordial free radicals that initiate the oxidative deterioration of lipids in muscle foods. Many reactions occurring routinely in vivo involve potent prooxidative states which are normally controlled by appropriate enzymes (Flohé, 1982). However, following slaughter, cutting, or exposure of meat tissues to air, such transient prooxidant states may no longer be controlled. We suggest that in fresh muscle foods, phagocytic cells may conceivably initiate and accelerate lipid oxidation.

The process of phagocytosis is associated with a dramatic burst of oxidative metabolism by phagocytic cells (neutrophils, eosinophils, monocytes, and macrophages) (Sbarra and Karnovsky, 1959). Thus, during ingestion of particulate materials (microorganisms, dead cells, oil drops, etc.) the consumption of oxygen is enhanced 10–15-fold within a few seconds after contact with the stimulating substances (Sabarra and Karnovsky, 1959; Takanaka and O'Brien, 1979). Hydrogen peroxide is concurrently produced in large amounts and accumulates in the medium surrounding the phagocytes (Iyer et al., 1961; Root et al., 1975), especially when its enzymatic degradiation is inhibited (Nakagawara et al., 1981). Phagocytic leukocytes also generate large amounts of superoxide anion radical during particle ingestion, and this is released into the medium surrounding the phagocytes (Babior et al., 1973; Johnston et al., 1975). The superoxide is most likely an intermediate in the formation of hydrogen peroxide by phagocytic cells (Root and Metcalf, 1977).

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Lipid peroxidation occurs during phagocytosis as indicated by a considerable loss of arachidonic acid (Shohet et al., 1974). These authors suggested that hydroxyl radicals (HO·) were the possible initiating factors in this oxidation. The initiation of lipid peroxidation in model systems by oxygen free radicals has been described by several researchers (Fong et al., 1973; Kellogg and Fridovich, 1975; Svingen et al., 1978; Cohen and Sinet, 1980; Fridovich and Porter, 1980) and reviewed by Tien et al. (1981). It is conceivable that granulocytes and monocytes in the blood of muscle tissues of animals, avian, and fish species can contribute to lipid oxidation and subsequent deterioration.

The highly reactive products of oxygen, namely, superoxide anion (O_2^{-}·), hydrogen peroxide (H_2O_2), the hydroxyl radical (HO·), and singlet oxygen ($^1O_2$) have been implicated as microbiocidal agents in the phagocytic cells (Gabig and Babior, 1981). The microbiocidal activity of H_2O_2 is considerably enhanced by myeloperoxidase (MPO) in the presence of a halide ion (I^- or Cl^-) (Klebanoff, 1975; Babior, 1978; Klebanoff and Clark, 1978). During the reaction of MPO with chloride or other halides, chlorine, hypochlorite, and other halide equivalents are increased (Harrison and Schultz, 1976).

The blood of fish contain three main groups of leukocytes—the granulocytes (neutrophils, eosinophils, and basophils), monocytes, and lymphocytes (Stachell, 1971). The granulocytes contain myeloperoxidase which is involved in phagocytic and other protective functions.

In the current study we examined the activity of a myeloperoxidase-like enzyme isolated from fish leukocytes and assessed its capacity to initiate lipid deterioration in the presence of H_2O_2 and halides.

EXPERIMENTAL SECTION

Materials. Hydrogen peroxide (30%), sodium chloride, potassium bromide, sodium citrate, and the potassium mono- and diphosphate were purchased from Mallinckrodt, St. Louis, MO. The β-carotene, pyrogallol, purpu-

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