Identification of the Most Intense Odor Compounds Formed During Autoxidation of Methyl Linolenate at Room Temperature[1]

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The volatile compounds formed during autoxidation of methyl linolenate (MLe) at 22–24°C were analyzed by a technique which reveals the flavor compounds having the highest flavor units (ratio of the concentration to the odor threshold). After a reaction time of 48 hr, when ca. 20 mol percent of the MLe had been converted into hydroperoxides, *trans,cis*-2,6-nonadienal followed by 1,*cis*-5-octadien-3-one, *trans,cis*-3-5-octadien-2-one and *cis*-3-hexenal showed the highest flavor units. After 102 hr 1,*cis*-5-octadien-3-one was by far the most important odor compound, followed by *cis*-3-hexenal and *trans,cis*-2,6-nonadienal.

A great variety of volatile compounds are formed as minor products during autoxidation of unsaturated acyl lipids. Because of their odor and taste properties, they decisively influence the aroma of fat-containing foods (1-3).

The secondary autoxidation products arise in widely differing amounts (1-3); because their odor and taste thresholds are very different (4-8), it is difficult to identify those compounds which contribute most significantly to the off-flavors caused by lipid peroxidation.

The estimation of flavor units (FU) was the first approach to evaluating the significance of the volatile compounds in a particular food flavor (9, 10). The FU of a compound is defined as the ratio of its concentration to its odor threshold.

However, the determination of FU is very laborious because concentration and threshold data must be determined for a great number of volatiles. For this reason, FU are known only for the flavor compounds of a small number of foods. One example is butter with a trainy off-flavor for which Badings (4) identified *cis*-4-heptenal, *trans,cis*-2,6-nonadienal and 1-octen-3-one as compounds with the highest FU.

Such painstaking determination of FU can be avoided by estimating a relative value such as the flavor dilution factor (FD factor) (11, 12). This factor is proportional to the FU value of each flavor compound in a mixture (12) and also allows a differentiation of the main flavor compounds from the background flavor components. The term "FD factor" replaces our previously used "D-value" to avoid confusion with uses of D-value in other disciplines.

The determination of the FD factors of volatile compounds is performed by a gas chromatographic analysis and effluent sniffing of a dilution series of the original aroma extract (11, 12). This procedure is less time consuming than the estimation of the FU and indicates the potent odor compounds which should be identified.

The new method was applied to the volatiles formed during autoxidation of linoleic acid and methyl linoleate (12). Hexanal, *cis*-2-octenal and *trans*-2-nonenal exhibited the highest FD factors for both lipids. 1-Octen-3-ol followed in the case of linoleic acid and 1-octen-3-one in that of methyl linoleate.

The aim of our investigation was to identify the most important odor compounds that contribute to the flavor produced during peroxidation of methyl linolenate (MLe) at room temperature.

EXPERIMENTAL

Materials. The following chemicals were used: MLe (at least 99% purity) from Sigma Chemical Co. (Munich, West Germany); *cis*-3-hexenol and *trans*-2-pentenal from EGA (Steinheim, West Germany); 1-penten-3-one, *trans*-2-hexenal, and *trans,trans*-2,4-heptadienal from Atlanta (Heidelberg, West Germany); *trans,cis*-2,6-nonadienal from Haarmann and Reimer (Holzminden, West Germany). 9-Hydroperoxy-*trans,cis,cis*-10,12,15-octadecatrienoic acid (9-HPOT) was a gift from G. Laskawy (DFA Garching, West Germany), and silica gel (0.063-0.2 mm; Merck, Darmstadt, West Germany) was washed with concentrated HCl (13) and its water content adjusted to 5.7% (w/w).

cis-3-hexenal was prepared by oxidation of *cis*-3-hexenol with pyridinium chlorochromate (14); its ^{1}H-NMR (200 MHz, $CDCl_3$) spectra was: α 0.99 (t, C6, 3H), 2.06 (m, C5, 2H), 3.19 (d, C2, 2H), 5.51 (dtt, J 10.6 Hz, C3, 1H), 5.71 (dtt, J 10.6 Hz, C4, 1H), 9.67 (t, J 2.1 Hz, C1, 1H). 1H). 1,*cis*-5-octadien-3-ol was prepared by a Grignard reaction of vinyl magnesium bromide with *cis*-3-hexenal (7, 15); the alcohol was then oxidized to 1,*cis*-5-octadien-3-one with pyridinium chlorochromate (7, 16). The ^{1}H-NMR data of the vinyl ketone agreed with that published by Swoboda and Peers (7).

Ethyl-2-chlorovinyl ketone was prepared from acetyl chloride and acetylene (17, 18); its reaction with diethylamine yielded methyl 2-(diethylamino)vinyl ketone (19); the latter compound was converted into *trans,cis*-3,5-octadien-2-one by reaction with 1-bromobut-1-ene in the presence of lithium (20); after purification by gas chromatography, the IR spectrum of the octadienone agreed with that published by Naef and Decorzant (20).

A mixture consisting of *trans,cis,cis*-2,4,7-decatrienal and *trans,trans,cis*-2,4,7-decatrienal was prepared by degradation of 100 μmol 9-HPOT with 25 μmol ascorbic acid under anaerobic conditions (21); after 60 min at room temperature, the pH of the reaction system was adjusted to 8.5 with NaOH and the aldehydes were extracted with pentane. After separation by capillary GC on C30W, the *trans,cis,cis*-2,4,7-decatrienal showed a rancid, oily odor and the *trans,trans,cis*-2,4,7-isomer a fatty, green odor at the sniffing port.

Autoxidation. A solution of 300 mg MLe in 3 ml diethyl ether was pipetted into a 250-ml Erlenmeyer flask. The solvent was removed under a stream of oxygen. The sealed flask was stored in the dark for 48 hr or 102

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hr at 22–24°C. After cooling the flask for 2 hr at -18°C, the reaction mixture was dissolved in 4 ml diethyl ether.

Isolation of the volatiles. After autoxidation, the reaction mixture was divided into two portions. Each portion was applied to a column (40 × 1.7 cm) packed with Silica gel 60 suspended in pentane/diethyl ether (97.5:2.5, v/v). Step by step elution was performed at 10°C with 150 ml of the same solvent mixture, then 100 ml of pentane/diethyl ether (95:5, v/v), 100 ml of pentane/diethyl ether (90:10, v/v) and finally 50 ml of pentane/diethyl ether (80:20, v/v). The volatile fraction between 200 and 400 ml was collected and concentrated (12) for analysis by capillary GC.

In order to obtain enough material for the identification experiments, the volatile fractions from three autoxidation runs were combined and rechromatographed on the Silica gel column described above. Step by step elution was performed as before, except that 100 ml of pentane/diethyl ether (80:20, v/v) were used as the last eluent. The effluent was collected in five fractions (90 ml each). Fractions 1 and 2 were discarded and each of the fractions 3–5 were concentrated (12) and then analyzed by capillary GC-MS.

Instrumental analysis. Capillary GC (HRGC) was carried out as previously described (12). The thin-film capillary columns used were capillary SE54 and capillary C30W. Retention data of the compounds are presented as retention indices (RI) according to Van den Dool and Kratz (22).

HRGC-MS analyses were performed using an MS8230 (Finnigan, Bremen, West Germany) in tandem with the SE54 and C30W capillaries. The conditions for the HRGC, the measurement of the electron impact mass spectra MS (EI) and chemical ionization spectra MS (CI) were the same as used in (12).

Peroxides. Peroxides were quantified with the FE test (23). The absorbances measured were referred to a calibration curve obtained with linoleic acid hydroperoxides.

HRGC eluate sniffing. The FD factors of the volatile compounds were determined as previously described (12). The odor threshold values were approximated by an olfactometric HRGC method (12) using *trans*-2-nonenal instead of hexanal as internal standard; the HRGC was performed on the capillary C30W.

RESULTS

Yield of volatiles. The volatiles were separated from the autoxidized MLe by chromatography on silica gel. In preliminary model experiments this procedure was optimized until the unreacted MLe was completely separated in the first 200 ml of the effluent and the volatile compounds appeared predominantly in the elution range 200 to 400 ml.

The data in Table 1 show that this chromatographic procedure resulted in a high recovery of ketones and aldehydes having 6 to 8 carbon atoms. The recovery of compounds with shorter and longer carbon chains was lower, because these compounds were either not completely separated from the MLe (this was found for the nonadienal) or remained partly or completely bound to the column, like the hydroperoxide fraction. Hence, the yields of 1-penten-3-one and of *trans*-2-pentenal were lowered in comparison to those of the C_6- to C_8-compounds and 1,*cis*-5-octadien-3-ol was not found at all.

TABLE 1

Yields of Volatiles after Isolation from a Model Mixture[a] by Column Chromatography

Compound	Yield[b] (%)
1-penten-3-one	53
trans-2-pentenal	67
cis-3-hexenal	84
trans-2-hexenal	87
1,*cis*-5-octadien-3-one	95
1,*cis*-5-octadien-3-ol	0
trans,trans-2,4-heptadienal	91
trans,cis-3,5-octadien-2-one	34
trans,cis-2,6-nonadienal	37

[a] The model mixture consisted of 300 mg MLe and 0.25 mg of each volatile compound. After isolation of the volatile fraction by column chromatography on Silica gel 60, methyl nonanoate was added as internal standard for the quantification of the volatile compounds by HRGC on the C30W capillary.

[b] Mean values from duplicates.

Separation of the volatiles from autoxidized free linolenic acid by chromatography on aluminum oxide (12) resulted in relatively high yields which were independent of molecular weight (data not shown). In spite of this, this procedure is not recommended because it is so drastic that degradation of secondary peroxides can occur.

Identification of flavor compounds. In order to identify the volatile compounds, MLe was autoxidized for 102 hr. During this time approximately 70 mol percent of the MLe were converted into peroxides.

The volatile fraction isolated by silica gel chromatography was analyzed by HRGC. During this procedure seven aroma-active regions were perceived at the outlet of the capillary column. Their positions in the gas chromatogram are shown in Figure 1.

The identification experiments focussed especially on the compounds which caused the seven odor impressions. As shown in Table 2, the following six odorants were identified: 1-penten-3-one; *cis*-3-hexenal; 1,*cis*-5-octadien-3-one; a 2,4-heptadienal; *trans,cis*-3,5-octadien-2-one and *trans,cis*-2,6-nonadienal. The seventh odorant (No. 12) showed a molecular weight of 138. Its MS (EI) and MS (CI) were identical with those of the *trans,trans*-2,4- and *trans,cis*-2,4-nonadienal but its RI did not agree with the retention data of the two nonadienals on the SE54 and C30W capillary columns.

The majority of the compounds which appear in Figure 1 were odorless because their concentrations in the effluent of the GC capillary in relation to their odor thresholds were too low. As shown in Table 2, 2-pentenal (probably the *cis*-isomer); *trans*-2-pentenal; *trans*-2-hexenal; *trans,trans*-2,4-heptadienal; a 3,5-octadien-2-one, and a pentenylfuran belonged to this group of background volatiles which at best modify somewhat the aroma note of the potent odor compounds. The *trans,cis,-cis*-2,4,7- and the *trans,trans,cis*-2,4,7-decatrienals, which have been identified as autoxidation products of linolenic acid (4), were not detected either in the MLe samples or in autoxidized free linolenic acid (data not shown).

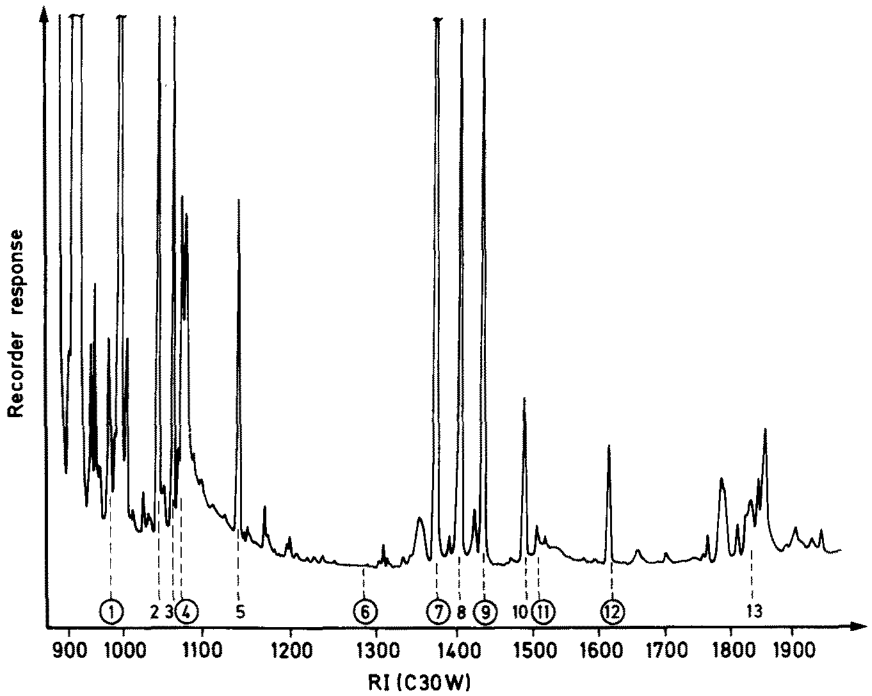

FIG. 1. Capillary GC of the volatile fraction from autoxidized methyl linolenate (22–24°C, 102 hr). The numbers at the bottom of the peaks refer to the compounds listed in Table 2. The numbers in circles indicate the positions where an odor was perceived at the sniffing port.

TABLE 2

Volatile Compounds Formed during Autoxidation of MLe for 102 hr at 22–24°C in the Dark

No.	Compound	RI SE54	 C30W	Aroma description
1	1-penten-3-one[a]	680	973	sharp, pungent
2	2-pentenal[b]	738	1043	---[c]
3	*trans*-2-pentenal[a]	749	1061	---[c]
4	*cis*-3-hexenal[a]	795	1072	green, apple-like
5	*trans*-2-hexenal[a]	851	1139	---[c]
6	1,*cis*-5-octadien-3-one[a]	984	1282	metallic, geranium-like
7	2,4-heptadienal[b]	1000	1373	fatty, nutty
8	*trans,trans*-2,4-heptadienal[a]	1012	1401	---[c]
9	*trans,cis*-3,5-octadien-2-one[a]	1095	1432	fatty, fruity
10	3,5-octadien-2-one[b]	1107	1487	---[c]
11	*trans,cis*-2,6-nonadienal[a]	1151	1506	green, cucumber-like
12	Unknown (M_r: 138)	1200	1611	fried fat
13	Pentenylfuran[b]	1289	1821	---[c]

[a] The compound was identified by comparing it with the reference substance on the basis of the following criteria: RI on the two capillaries (SE54 and C30W) of different polarity, mass spectra obtained by MS (EI) and MS (CI) and odor quality.
[b] The peak was identified by comparison with data from the library of mass spectra.
[c] Odorless at the sniffing port.

FD-factor determination. The FD factors of the seven flavor compounds were determined in MLe samples which were autoxidized for 48 hr and 102 hr.

After 48 hr ca. 20 mol percent of the MLe had been converted into hydroperoxides. The data (corrected for yield losses) listed in Table 3 show that *trans,cis*-2,6-nonadienal followed by 1,*cis*-5-octadien-3-one showed the highest FD factors. In addition to these, two compounds which were the most important odor compounds at this autoxidation stage, *trans,cis*-3,5-octadien-2-one and *cis*-3-hexenal, contributed to the odor but with lower FU. On the basis of their low FD factors, the unknown (M_r 138), 1-penten-3-one and the 2,4-heptadienal were of lesser importance for the overall aroma.

After 102 hr 1,*cis*-5-octadien-3-one was by far the most important odor compound (Table 3). Its FD factor (corrected for yield losses) was 24to 34-fold higher than those of the next important odor compounds, *cis*-3-hexenal and *trans,cis*-2,6-nonadienal.

The autoxidized free acid showed essentially the same ranking of the most potent odor compounds (data not shown). The only difference was that 1,*cis*-5-octadien-3-ol, isolated by chromatography on aluminium oxide, was also found in the product mixture. However, on the basis of its relative low FD factor this alcohol was of minor importance to the oxidation flavor.

Approximate odor threshold values. The determination of odor thresholds of volatiles arising from the peroxidation of lipids by an olfactometric technique has been performed only for a relatively small number of volatiles (24). These data do not include the compounds identified in this study as important flavor compounds. Therefore their thresholds were approximated by the olfactometric HRGC method.

The data summarized in Table 4 show that the lowest odor threshold value (ca. 0.07 ppb, v/v) was found for 1,*cis*-5-octadien-3-one. This value is approximately 25- and 35-fold lower than those for *cis*-3-hexenal and *trans,cis*-2,6-nonadienal, respectively. 1-Penten-3-one showed the highest odor threshold of the four compounds investigated.

TABLE 3

FD Factors of the Flavor Compounds Isolated after Autoxidation of MLe for 48 hr and 102 hr at 22–24°C

No.[a]	Compound	FD-Factor[b] 48 hr		FD-Factor[b] 102 hr	
1	1-penten-3-one	<1[c]		4	(7)
4	*cis*-3-hexenal	4	(4)	12	(14)
6	1,*cis*-5-octadien-3-one	16	(16)	340	(340)
7	2,4-heptadienal	<1[c]		4	(4)
9	*trans,cis*-3,5-octadien-2-one	2	(6)	2	(6)
11	*trans,cis*-2,6-nonadienal	16	(41)	4	(10)
12	Unknown (M_r: 138)	2		4	

[a] As in Table 2.

[b] The FD factor corrected for the differences in the yield losses is given in brackets; the corrections were made on the basis of 1,*cis*-5-octadien-3-one according to the yields listed in Table 1.

[c] FD factor <1 means the compound was odorless at the sniffing-port.

TABLE 4

Odor Threshold Values

Compound	Approximate threshold range[a] (ppb, v/v)
1-penten-3-one	3.7 -10.2
cis-3-hexenal	1.0 - 2.7
trans,cis-2,6-nonadienal	1.4 - 3.9
1,*cis*-5-octadien-3-one	0.04- 0.1

[a] The odor thresholds were approximated using *trans*-2-nonenal as internal standard for which Hall and Andersson (24) determined by dynamic gas dilution olfactometry an odor threshold of 1.2 ppb (v/v). The threshold range was established by the lowest and the highest values found in triplicates.

DISCUSSION

The results reveal that autoxidation of MLe at room temperature leads to seven predominant odor compounds: 1,*cis*-5-octadien-3-one; *trans,cis*-2,6-nonadienal; *cis*-3-hexenal; 1-penten-3-one; 2,4-heptadienal; *trans,cis*-3,5-octadien-2-one, and an unknown substance. The ranking of their odor intensity was dependent on the oxidation stage of the MLe. After a reaction time of 48 hr, *trans,cis*-2,6-nonadienal was the most important followed by 1,*cis*-5-octadien-3-one. Prolonged autoxidation leads to an increase in the aroma intensity especially of the 1,*cis*-5-octadien-3-one. Its geranium-like odor note was now perceived in the odor profile of the MLe but its concentration was so low that no peak appeared in the gas chromatogram. Only after enrichment was it detectable by HRGC and by mass spectrometry.

The threshold data found with the olfactometric procedure confirm that from all volatile compounds which are formed by peroxidation of unsaturated lipids, the 1,*cis*-5-octadien-3-one showed the lowest odor threshold. Its value and that of the *trans,cis*-2,6-nonadienal were found to be in a range similar to those reported for their solutions in oil (5, 7). This agreement of the results is unexpected; actually the threshold value found by an olfactometric procedure, in which all of the material is volatilized and therefore smelled, should be lower than that of the compound in solution, where it is distributed between the gaseous phase and the oil. This difference was found for *cis*-3-hexenal: the threshold value obtained by the olfactometric method was 60 times lower than when dissolved in oil (6).

In the autoxidized MLe, the metallic, geranium-like odor of the 1,*cis*-5-octadien-3-one was probably rounded off by other carbonyl compounds to a fully oxidized off-flavor. Such an effect was observed by Hammond and Hill (25) in the case of another vinyl ketone, the 1-octen-3-one. Its metallic flavor in milk was modified to an oxidized flavor by addition of small amounts of octanal, 2-heptenal and 2,4-heptadienal.

The identification of 1,*cis*-5-octadien-3-one confirms the hypothesis of Hammond and Hill (25) that stated that the formation of this vinyl ketone might account for the oxidized-metallic aroma note of autoxidized MLe (26).

Swoboda and Peers (7) were the first who identified the 1,*cis*-5-octadien-3-one and described its sensory properties. They found that it was responsible for a metallic off-flavor in caramels and revealed its formation when butterfat was autoxidized in the presence of α-tocopherol and cupric palmitate. Two ω-3 fatty acids with five and six double bonds which occurred in the butterfat were identified as precursors of the 1,*cis*-5-octadien-3-one (27). Because linolenic acid (also an ω-3 fatty acid) did not produce the vinyl ketone, Swoboda and Peers (28) postulated that the vinyl ketone could only be formed by ω-3 fatty acids containing four or more double bonds. Our results actually show that the vinyl ketone is also formed from linolenic acid; this demonstrates that an ω-3 fatty acid containing three double bonds also can be a precursor of 1,*cis*-5-octadien-3-one.

The 1,*cis*-5-octadien-3-one is obviously an oxidation product of the 1,*cis*-5-octadien-3-ol (Fig. 2) whose formation can be explained analogously to that of 1-octen-3-ol, an autoxidation product of linoleic acid (29). It has been postulated that this allyl alcohol results from a β-scission of the 10-hydroperoxide isomer of linoleic acid (3). This is supported by the studies of Frankel *et al.* (30), who found that the hydroperoxide isomers obtained from methyl linoleate decompose, among others, into 1-octen-3-ol and 10-oxo-8-decenoic acid. Analogous to this pathway we propose that the 1,*cis*-5-octadien-3-ol arises from a β-scission of the 10-hydroperoxide of methyl linolenate (Fig. 2). It should be investigated whether small amounts of this hydroperoxide are formed in the dark by an oxidation of the mono-allylic carbon-8 of MLe. Such side reaction was detected in the case of linoleic acid (31). After photosensitized oxidation of MLe, the 10-hydroperoxide is one of the major products (30, 32).

The formation of the other two odor compounds of major importance also has been explained by a β-scission of hydroperoxide isomers. The 13-isomer was suggested as precursor for *cis*-3-hexenal (1, 4) and the 9-isomer for *trans,cis*-2,6-nonadienal (25). Experiments in which either a hydroperoxide mixture containing these isomers or the individual positional isomers of methyl linolenate hydroperoxides were thermally decomposed support these assumptions (30, 33).

The significance of these potent odor compounds, which originate from autoxidized linoleic and linolenic acids, to the off-odor of stored soybean oil is being investigated.

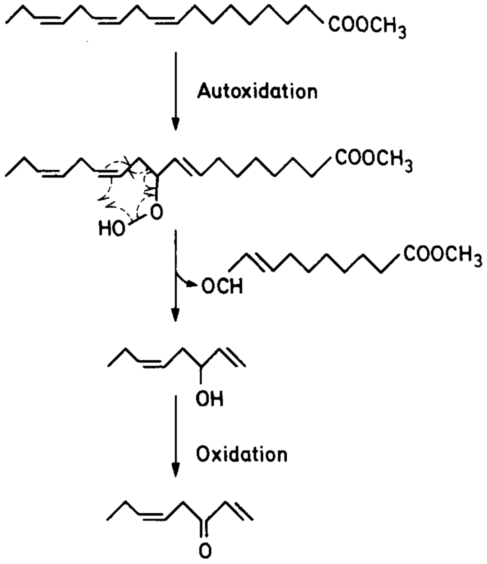

FIG. 2. Hypothetical pathway for the formation of 1,*cis*-5-octadien-3-one during autoxidation of methyl linolenate.

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