# Technical

## Volatile Components from Trilinolenin Heated in Air[1]

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### ABSTRACT

Headspace volatiles derived from trilinolenin heated to 192 C in air were collected, separated and identified using a microroom/GC-MS/computer system. Data from four analyses indicated more than 80 volatiles were present. Of these volatiles, 38 have been identified, and their combined GC peak areas represent 90% of the total integrated chromatographic area. Dominant volatiles identified included: ethanal (2%), 2-pentene (3%), ethanol (0.5%), 2-propenal (20%), propanal (7%), ethyl furan (13%), 2-butenal (4%), 2-pentenal (4%), 2,4-heptadienal (21%), 4,5-epoxy-2-heptenal (3%) and 2,4,7-decatrienal (2%). Most of the dominant and some of the minor volatiles are the predicted decomposition products of the four linolenin monohydroperoxides. However, the ratios of the predicted volatiles do not correspond to the monohydroperoxide ratios observed from autoxidized linolenate. Other primary and minor volatiles are present that are not predicted from the classical hydroperoxide decomposition hypothesis, which suggests that trilinolenin and some of the volatile compounds undergo further oxidation.

### INTRODUCTION

It is well known that soybean oil (SBO), which contains ca. 8% linolenic acid (Ln), readily oxidizes and produces objectionable volatile compounds (1,2). Although each major fatty acid of SBO is a potential precursor for those volatiles (3-6), Ln oxidizes at the faster rate (7,8) and is ascribed to be the primary contributor to SBO organoleptic deficiencies (2), especially when subjected to cooking oil temperature (9,10).

Various studies have shown that oxidized Ln forms four (from ground state oxygen) or six (from singlet oxygen) monohydroperoxides (11,12). It is the decomposition of those hydroperoxides to volatile compounds that probably are, at least in part, responsible for the objectionable odors and flavors of oils containing Ln (13,14).

There are numerous investigations on volatiles derived from the trienoic acid; however, only a few include Ln oxidized at or near cooking oil temperature. Gaddis et al. (3) isolated the monocarbonyls from methyl linolenate oxidized at 25 C and then heated to 165 C; they identified 2,4-hepta- and nonadienals as the predominant volatiles. They also reported a trace of an unidentified trienal. Frankel et al. (11) isolated pure monohydroperoxides from auto- (27 C) and photosensitized (0 C) oxidized linolenate and found that a decomposition temperature of 210 C produced the same volatiles; however, autoxidized Ln produced more ethane and decatrienal, whereas photosensitized oxidation resulted in more 2-butenal. Staph and Daubert (9) identified three aldehydes via their dinitrophenylhydrazones (DNPH) from unhardened SBO oxidized at 200 C. The aldehydes reported are associated with oxidized oleic and linoleic acids and not linolenic acid. Hoffmann (13) also used the DNPH methodology to isolate volatiles from SBO heated at 120 C. He observed 3-hexenal and 2,4-hepta- and octadienals and concluded that the precursor of these compounds was Ln. The most detailed study on oxidation of Ln at elevated temperatures was reported by Lomanno and Nawar (15). They subjected ethyl linolenate to temperatures of 70, 180 and 250 C in air and isolated and identified a number of volatile compounds containing different functional groups. Those compounds included hydrocarbons, alcohols, aldehydes, furans and ethyl esters. Conspicuously absent was decatrienal.

This report on characterization of volatiles from heated trilinolenin is a continuation of our research to identify compounds derived from SBO when heated to cooking oil temperature. For our studies, we chose to use as model systems pure triglycerides of each major fatty acid of SBO. This approach lends to the identification of the less concentrated constituents that still may be important organoleptically and also facilitates fatty acid precursor assignments. Our previous reports described volatiles observed from tristearin (4), triolein (5) and trilinolein (6).

### EXPERIMENTAL

Trilinolenin was prepared from glycerine, linolenic acid and an esterification catalyst, *para*-toluenesulfonic acid, according to Wheeler et al. (16). The synthesized triglyceride was purified by liquid solid (silica gel) chromatography.

Fatty acid composition [by gas chromatography (GC)-packed column; 6 ft x 4 mm id, 10% EGSS-X and capillary column; 50 m x 0.25 mm, OV-275] was as follows: linolenic acid >99% with less than 0.1% each of palmitic, stearic, oleic and linoleic acids. The triglyceride contained 1.2% conjugated diene (calculated from absorption at 233 nm) and 0.07% conjugated triene (calculated from absorption at 268 nm) (17). There was no indication of free fatty acids and mono or diglycerides [by TLC, Kieselgel 60 F-254 precoated (0.25 mm thick) silica gel TLC plates developed in petroleum ether/diethyl ether/acetic acid mixture, 70:25:5].

Three other oil samples were procured for additional heated oil/volatile analysis. The analyses are only partially completed and include GC peak area integrations and a few compound identifications, namely, acrolein (see Discussion). Of the three samples, soybean and menhaden oils were purchased as bleached, deodorized and refined oils, which were stored at -18 C until needed. The third sample, a triacylglyceride composed of all (>99%) *cis,cis*-9,15-octadecadenoic acid, was synthesized, purified and analyzed as reported previously (18). Each of the oils was deodorized at 4 mm pressure for 1.5 hr at 185-205 C just prior to use. Volatile analysis procedure of these three samples and of trilinolenin was identical and is described below.

Specific details of the volatile collection and analysis procedure have been reported (4,5). Briefly, 2 cm$^3$ of trilinolenin was heated to 192 C in a stainless steel container that we frequently call a "microroom." During the subsequent 10-min heating period, volatiles generated from the hot oil were continuously swept out of the container with air and collected directly on a GC column (glass, 14 ft x 4 mm id, packed with 10% OV-17 on chromosorb G) cooled to -60 C. Subsequently, the volatiles were separated by temperature programming (nonlinear from -60 C to 0 C, then 2 C/min to 250 C). As volatiles eluted from the GC column, they were simulataneously

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[1] Presented at the 73rd annual AOCS meeting, Toronto, 1982.

monitored by a flame ionization detector (FID), by sniffing at the GC exit port, and by a mass spectrometer (MS) (Nuclide 12-90 double focusing, magnetic scanning) set to scan from M/Z 10 to 450 every 9 sec. The GC was coupled to the MS via a silicon rubber membrane separator. Output from the MS was stored in a computer system for later processing. Mass spectrometric identifications of volatile compounds, matched by computer and manually with those of our Center's library of 35,000 mass spectra of known compounds (mass spectra of known compounds were obtained from the National Institute of Health, Bethesda, MD) were confirmed by GC retention data. The total volatile GC profile was obtained via a flame ionization detector. Areas under individual GC peaks were integrated and calculated as relative percent GC peak areas by a computer system, which has been described previously (4).

Four replicate trilinolenin volatile analyses were performed using a different sample each time. The four sets of data on integrated GC peaks were combined and averaged.

## RESULTS

More than 80 volatile compounds were formed from trilinolenin heated to cooking oil temperature (192 C) in air. The GC profile of those volatiles is shown in Figure 1, and compound identifications with corresponding integrated GC peak areas are listed in Tables I and II. The data listed in these tables show that 23 dominant compounds contributed 87% to the total integrated area of the gas chromatogram, that 15 minor constituents accounted for 4.0% more, and that 42 unknowns added an additional 9.0% to the total area. As mentioned, the percentages are an average of four separate volatile analyses and have an overall standard deviation of ± 15%.

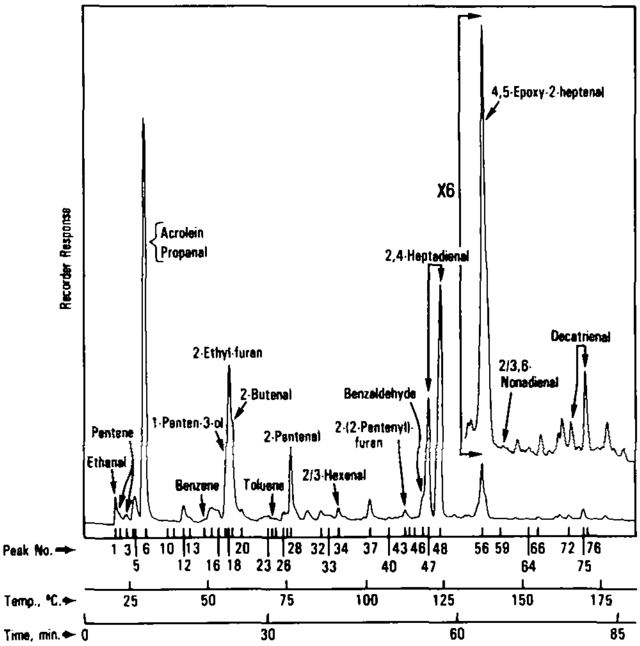


FIG. 1. Gas chromatograph (FID) of volatile compounds from thermally oxidized trilinolenin. Numbers 1-75 refer to GC peaks in the curve.

A number of GC peaks were only partially resolved, however, with exceptions listed below; most were sufficiently separated to allow computer integration of the individual GC areas. Acrolein and propanal (GC peak 6) eluted at nearly the same time, but they were fractionated to the extent that each could be identified from their mass spectra. According to mass spectra data (project 44) from the American Petroleum Institute (19), both aldehydes produce parent ions (acrolein M/Z) = 56 and propanal M/Z = 58) that have nearly the same sensitivities (parent ion peak height per micron of compound). Additionally, their parent ions are free from interference from the others' mass spectra. Consequently, the total GC area (peak 6) was apportioned according to the ratios of the observed parent

**TABLE I**

**Dominant Volatiles from Heated Trilinolenin**

| | | Trilinolenin | Methyl linolenate[a] hydroperoxides | | Ethyl linolenate[b] |
|---|---|---|---|---|---|
| Peak no. in GC (Fig. 1) | Compound | 192 C Air relative % | Autoxidized (27 C) relative % | Photosensitized (0 C) relative % | 180 C Air observed |
| 1 | Ethanal | 1.8 | 0.8 | 0.6 | |
| 6 | 2-Propenal (acrolein) | 19.7 | 5.8 | 6.7 | + |
| 6 | Propanal | 7.0 | 1.9 | 2.3 | + |
| 12 | Butanal | 1.3 | 0.1 | 0.8 | |
| 19 | 2-Butenal | 4.4 | 0.5 | 11.0 | + |
| 26, 28[c] | 2-Pentenal | 4.2 | 1.6 | 1.2 | + |
| 34 | 2/3 Hexenal | 0.6 | 1.4 | 3.4 | + |
| 47, 48[c] | 2,4-Heptadienal | 20.8 | 9.3 | 8.8 | + |
| 56 | 4,5-Epoxy-2- heptenal | 2.9 | 0.2 | 0.2 | |
| 72, 75[c] | 2,4,7-Decatrienal | 1.4 | 14.0 | 4.8 | |
| 2, 3[c] | Pentene | 2.9 | | | + |
| 5 | Furan | 2.2 | | | |
| 13 | Formic acid | 0.6 | | | |
| 17 | 1-Penten-3-ol | 0.5 | | | |
| 18 | 2-Ethyl furan | 12.9 | | | + |
| 20 | Ethyl vinyl ketone | 1.2 | | | |
| 37 | 2-Butyl furan | 1.4 | 0.5 | 0.3 | |
| 43 | 2-(2-Pentenyl) furan | 0.6 | | | + |
| 46 | Benzaldehyde | 1.1 | | | + |
| | Subtotal | 87.5 | 36.1 | 40.1 | |

[a] Reference 11.

[b] Reference 15.

[c] GC peak sets 26 and 28; 47 and 48; 72 and 75, plus 2 and 3 appear to be the result of geometric isomers.

+Indicates compounds those authors observed.

**TABLE II**

**Minor Volatiles from Heated (192 C) Trilinolenin**

| GC Peak no. | Compound | Relative % |
|---|---|---|
| 16 | Acetic acid | 0.9 |
| 23 | Propionic acid | 0.3 |
| 4 | Ethanol | 0.5 |
| 10 | Propanol | 0.1 |
| 40 | 2,4-Hexadienal | 0.2 |
| 59 | 2,6-Nonadienal[a,b] | 0.2 |
| 11 | 2-Methyl furan[c] | 0.1 |
| 25 | 2-Propyl furan[c] | 0.1 |
| 14 | Benzene | trace |
| 24 | Toluene | 0.4 |
| 32, 33 | Xylene | 0.5 |
| 45 | Methyl styrene | 0.3 |
| 64 | Trichlorobenzene | 0.1 |
| 66 | Naphthalene | 0.3 |
| 76 | Methyl napththalene | trace |
| | Unknowns (42) | 8.5 |
| | Subtotal | 12.5 |

[a] Compound also observed from auto- and photosensitized oxidized methyl linolenate.

[b] Believe compound originally was 3,6-nonadienal, that isomerized under experimental conditions – see text.

[c] Tentative identifications.

ions. Areas for GC peaks 25 and 26 [2-propyl furan and *cis*-2-pentenal] and peaks 43 and 44 [2-(2-pentenyl) furan and and unknown] were manually integrated. Single ion monitoring (5) was used to locate the concentration apex in the GC curve for each of those four compounds. That information was used to determine where verticals should be placed on each of the partially resolved GC peaks for apportionment and manual integration.

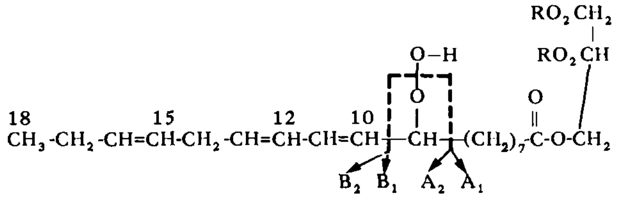
Both GC peaks 2 and 3 were attributed to pentene. The mass spectrum from peak 3 matches that of 2-pentene, an anticipated volatile from Ln 13-hydroperoxide. Peak 2 is attributed to an unknown pentene isomer(s). Three compounds, 2-pentenal (peaks 26 and 28), 2,4-heptadienal (peaks 47 and 48), and 2,4,7-decatrienal (peaks 72 and 75) are each associated with two sets of GC peaks consisting of a small peak followed by a large one. Previous studies (5,6) indicate that these sets of peaks are the result of GC separation of *cis* and *trans* isomers, with the small peak associated with a *cis* double bond and the large one with only *trans* double bonds.

Two unusual compound were identified from their mass spectra. These compounds were 2-(2-pentenyl) furan (peak 43) and 4,5-epoxyhept-2-enal (peak 56). The furan was postulated by Smagula, Ho and Chang (20) to be a possible volatile from oxidized linolenate. Those authors synthesized the compound and published its mass spectrum, which was used for our MS identification for compound 42. Although we had no authentic compound available for GC elution temperature determination, we observed that pentenyl furan elutes 4 C higher than 2-pentyl furan. This temperature difference follows elution characteristics of our GC column for such isologous compounds. Swoboda and Peers isolated and identified 4,5-epoxyhept-2-enal as a major component from oxidized butter fat (21). Their published MS of the epoxyenal corresponded to that obtained from the compound producing GC peak 56. Swoboda and Peers postulated that part of the 16-monohydroperoxide formed from Ln converts to the 15,16-epoxy-12-hydroperoxy-13-ene configuration and that subsequent carbon-carbon cleavage between the 11 and 12 carbon atoms would yield 4,5 epoxyhept-2-enal. A homologue, 4,5-epoxy-2-decenal, was identified from trilinolein heated (192 C) in air (6). The allylic hydroperoxide epoxide, *trans*-12, 13-epoxy-9-hydroperoxy-*trans*-10-octadecenoic acid, expected from the 13-OOH of linoleic acid was suggested as the precursor.

GC peaks 34 and 59 were identified as 2-hexenal and 2,6-nonadienal, respectively. It is well known that unsaturated aliphatic normal aldehydes with a double bond beta to the aldehyde carbonyl undergo conjugation at the heated microroom or GC injection port temperatures (22). Since neither 2-hexenal nor 2,6 nonadienal are anticipated decomposition products from oxidized linolenic, it is assumed both conjugated aldehydes were originally 3-hexenal and 3,6-nonadienal.

## DISCUSSION

This study of volatiles from heated trilinolenin can provide a more comprehensive insight as to the type and relative quantities of volatile compounds producing off-odors and off-flavors in linolenate-containing oils used for deep-fat frying. The primary autoxidation ($^{3}O_{2}$) products of linolenic acid include the 9-OOH (32%), 12-OOH (11%), 13-OOH (10%) and 16-OOH (46%) conjugated monohydroperoxides (-OOH) (11,12). If singlet oxygen ($^{1}O_{2}$) (formed via photosensitized dye) is involved, then six monohydroperoxides are observed: the same four isomeric conjugated hydroperoxides–9-OOH (23%), 12-OOH (12%), 13-OOH (14%) and 16-OOH (25%), plus two non-conjugated hydroperoxides 10-OOH (13%) and 15-OOH (13%) (11). Compounds predicted from homolytic decomposition of each of these hydroperoxides by carbon-carbon cleavage on each side of the hydroperoxy carbon atom would yield from trilinolenin two glyceride bound fragments, a carbonyl compound and an alkyl radical. As an illustration, the conjugated 9-OOH on decomposition would fragment as follows:



$A_1$ = glyceride bound fragment, $A_2$ = 2,4,7-decatrienal, $B_1$ = glyceride bound fragment, and $B_2$ = a 9-carbon triene radical. Products $A_1$ and $B_1$ are nonvolatile and would not be observed. However, when methyl and ethyl linolenates are oxidized, the $A_1$ fragment (octanoate) and $B_1$ fragment (9-oxo-nonanoate) are the most abundant volatiles observed (11,15). Fragment $A_2$, 2,4,7-decatrienal (peaks 72 and 75 in Fig. 1) was identified in this and other studies involving oxidation of lipids containing linolenic acid (3,11,14,24). However, Gaddis et al. (3) and Lomanno et al. (15) found little or no 2,4,7-decatrienal from oxidized methyl linolenate. The amount (1.5%) we and other investigators observed is not reflective of the large relative quantity of the 9-OOH formed (23%). It therefore appears that 2,4,7-decatrienal may be subject to oxidation to form secondary oxidation products, e.g., propanal and 2-pentenal, as suggested by Frankel (25). Decatrienal is reported by Badings (24) and Meijboom and Stroink (14) possibly to contribute to the trainy or fishy flavor of SBO. Our odor description of the aldehyde as it eluted off the GC column varied from dank to painty. Perhaps, as mentioned by Badings (24), the trainy or fishy characteristic of the trienal is evident only when the compound is highly diluted. Although the predicted nonatriene hydrocarbon (from the $B_2$ cleavage) was not observed, in cases where the $B_2$ cleavage involves a saturated alkyl $\omega$ section of the fatty acid, the corresponding saturated hydrocarbon is noted. In the above illustration, supposedly, the 9-carbon triene hydrocarbon radical $B_2$ ($CH_3\text{-}CH_2\text{-}CH{=}\overset{15}{C}H\text{-}CH_2\text{-}CH{=}\overset{12}{C}H\text{-}CH{=}\overset{10}{C}H\cdot$) can interact with a hydrogen or hydroxyl radical to form nonatriene (not observed) or 3,6-nonadienal (by tautomerism), GC peak 59 in Figure 1, respectively. Parallel radical reactions have been proposed to account for pentane from linoleic's 13-OOH ($B_2$ cleavage between the 13 and 14 carbon-carbon bonds) (23) and decanal from oleic's 8-OOH ($B_2$ cleavage between the 8 and 9 carbon-carbon bonds) (26). Besides those two reactions, the 9-carbon triene radical may be further oxidized to form other volatiles. Despite the lack of confirmational evidence for the occurrence of the $B_2$ cleavage forming the triene radical, the above model is the best available based on our current knowledge of session mechanisms.

If the same hydroperoxide decomposition analogy is applied to the other linolenate hydroperoxides, the resulting volatiles (predicted and actually observed) are listed in Table III. Numbers in brackets show the relative percent values (GC peak areas) noted. Integrated GC peak areas of volatiles derived from triolein and trilinolein indicate that the aldehydes produced by $A_2$ cleavage are present in significant amounts (5,6). This observation is also apparent for compounds (2,4-heptadienal, 2-butenal and propanal) formed from the degradation of linolenic's 12-OOH, 15-OOH and 16-OOH, but not for compounds predicted from linolenic's 9-OOH, 10-OOH and 13-OOH (2,4,7-decatrienal,3,6-nonadienal and 3-hexenal)—see Table III. Perhaps the methylene-interrupted double bond(s)—the 7, the 3 and 6, and the 3 in decatrienal, nonadienal and hexenal, respectively—increase the susceptibility of these compounds to undergo secondary oxidation reactions compared to propanal or the other 2-enals. Also note in Table III that volatiles such as propanal, 2-propenal and 2,4-heptadienal potentially have more than one precursor, which may account for the larger quantities formed of these compounds. Under our 10-min volatile collection procedure, ethane (hydrocarbon formed from the 16-OOH) would not be observed. However, 10% ethane was noted when spontaneously produced by thermal degradation of methyl linolenates' hydroperoxides, which includes the 16-OOH (11).

Most compounds listed in Table III are observed as dominant volatiles from oxidized trilinolenin (Table I). Not only did we note those major volatiles, but they were also noted by Frankel et al. (11) from direct thermal decomposition of methyl linolenate monohydroperoxide mixture, as well as by Lomanno and Nawar (15) from heated and oxidized ethyl linolenate.

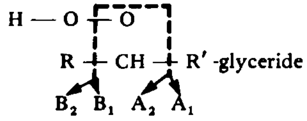
Data from Frankel et al. (11) were included in Table I to show that monohydroperoxides are precursors for many of the volatiles we observed, and that singlet oxygen, even at cooking oil temperature, may account for some of the 2-butenal (from the 15-OOH) observed. Since under our experimental conditions no photosensitized dye is involved, singlet oxygen if present must be produced by another method. Such a method—the Russell mechanism—is a plausible route (27). That method involves the interaction of two peroxy radicals to form a tetroxide dimer, which on decomposition gives oxygen as one of the products. Nakano et al. (28) provided spectroscopic evidence that singlet oxygen is obtained in self-reactions of linoleic acid peroxy radicals, and they suggested the Russell mechanism as the pathway. Note in Table I that autoxidized methyl linolenate produced 0.5% 2-butenal and that photosensitized oxidation (singlet oxygen) produced 11% 2-butenal. It may be argued that 2-butenal could be a secondary oxidation product (3-hexenal + $O_2 \rightarrow$ 2-butenal + ethanal) and that singlet oxidation is not viable at 192 C. However, in addition to the formation of 2-butenal from oxidized trilinolenin, the formation of 2-heptenal from $^1O_2$ oxidation of linoleic acid (11) plus the identification of 2-heptenal from heated (192 C) trilinolein (6) also suggest that singlet oxygen is involved in fatty acid oxidations.

In Table I, data from the Lomanno and Nawar study on the 180 C oxidation of ethyl linolenate in air (15) show they had reported many of the same major volatiles that we noted in this study. Of interest is the mutual observation of 2-(2-pentenyl) furan from oxidized linolenic acid. Their

**TABLE III**

**Linolenate Hydroperoxides**

Possible pathways in the formation of some volatiles from trilinolenin



| | Cleavage pattern | | |
|---|---|---|---|
| | $A_2$ | $B_2$ | |
| Hydroperoxide | | By addition of •H or •OH to HC radical | By further oxidation of $B_2$ or linolenic OOH |
| | | Autoxidation – $^3O_2$ | |
| 9-OOH | 2,4,7-Decatrienal (1.5%) | 3,6-Nonadienal (0.2%) | 2-(2-Pentenyl) furan (0.6%)<br>2,4-Heptadienal (20.7%) |
| 12-OOH | 2,4-Heptadienal (20.7%) | 3-Hexenal (0.6%) | 2-Ethyl furan (2.6%) |
| 13-OOH | 2-Hexenal (0.6%)[a] | 2-Pentene (2.9%) | 2-Pentenal (3.6%)<br>1-Penten-3-ol (7.7%)<br>Butanal (1.8%) |
| 16-OOH | Propanal (7.0%) | Ethane (?)<br>Ethanol (0.5%) | Ethanal (1.8%) |
| | | Singlet oxygen ($^1O_2$) – oxidized | |
| 10-OOH | 2,6-Nonadienal (0.2%)[b] | None observed | 2,4-Hexadienal (0.2%)<br>Propanal (7.0%) |
| 15-OOH | 2-Butenal (5.2%) | Propanal (7.0%) | 2-Propenal (19.6%) |

[a] Believe compound originally was 3-hexenal, that isomerized under experimental conditions – see text.
[b] Believe compound originally was 3,6-nonadienal, that isomerized under experimental conditions – see text.

**TABLE IV**

**2-Propenal (Acrolein) from Various Oils**

| | Fatty acid composition (relative %) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Relative % 2-propenal | Sat | Mono | Di | Tri | >Tri | Other |
| Tristearin[a] | 0.0 | 100.0 | – | – | – | – | – |
| Triolein[b] | 0.2 | 0.8 | 98.9 | 0.1 | 0.2 | – | – |
| 9c,15c-Triisolinolein | 0.0 | – | – | 99.8 | 0.2 | – | – |
| Trilinolein[c] | 4.2 | – | 0.1 | 99.8 | 0.1 | – | – |
| Soybean oil | 7.2 | 15.0 | 23.0 | 54.0 | 8.0 | – | – |
| Trilinolenin | 19.6 | 0.3 | 0.3 | 0.2 | 99.0 | – | – |
| Menhaden oil[d] | 31.2 | 37.0 | 26.0 | 3.0 | 1.0 | 28[e] | 5 |

[a] Reference 4.
[b] Reference 5.
[c] Reference 6.
[d] Reference 32.
[e] $C_{18}$:4 (3%), $C_{20}$:4 (2%), $C_{20}$:5 (13%), $C_{22}$:5 (2%), $C_{22}$:6 (8%).

studies and ours appear to be the only ones in which the furan was actually noted from an ω 3, 6, 9 unsaturated fatty acid. Smagula et al. postulated 2-(2-pentenyl) furan as a possible volatile from oxidized linolenate (20). Those authors synthesized the furan, published its mass spectrum, which we used for our MS identification, and evaluated its odor and flavor. They mentioned that the *trans* isomer at 4 ppm has a painty-metallic odor. We noted a metallic-sour-dank odor as the compound eluted off the GC column. Derivation of 2-(2-pentenyl) furan may follow similar paths as those mentioned by Sessa (29) for the formation of 5-(pentenyl)-2-furaldehyde from soy phospholipids by $^1O_2$ oxidation of the conjugated 9-OOH of linolenic acid.

In a similar manner, 2-ethyl furan can be derived from the $^1O_2$ oxidation of the conjugated (13,15) 12-OOH of linolenic acid or the $^1O_2$ oxidation of the conjugated (5,7) 4-OOH of decatrienal. Briefly, the $^1O_2$ oxidation (1,4 addition) of the conjugated diene portion of the OOH leads to a six-member cyclic peroxy ring, which on decomposition leads to the substituted (ethyl) furan (30,31). An alternative path for the formation of 2-ethyl furan is postulated by Grosch (32). The mechanism includes the $B_2$ cleavage of linolenics 12-OOH–see illustration–to produce a 1,3-hexadiene radical. The radical oxidizes to a hydroperoxide ($CH_3$-$CH_2$-CH=CH-CH=CH-OOH) which decomposes via an alkoxy radical and cyclizes to form 2-ethyl furan.

In Table II are listed the minor volatiles derived from oxidized trilinolenin that probably are related to secondary oxidation reactions. Results from GC integration show that the minor compounds contributed 12.5% to the total GC area and that the 42 unknowns accounted for 8.5% of that subtotal. Of the identified minor constituents, the aromatic compounds as a particular class of compounds are in the majority. Whether these minor aromatics are genuine products of trilinolenin decomposition of are contaminants (trichlorobenzene is assumed to be a contaminant) is not known.

During the course of identifying volatiles from various pure triglycerides, we noticed the increased quantity of 2-propenal with increased fatty acid unsaturation. This prompted us to reexamine our prior triglyceride/volatile data for the 2-alkenal quantity variation. In Table IV are listed the relative percents (GC peak area) of 2-propenal derived from six different samples heated (192 C) in air. Those results show that saturated, mono- or diunsaturated fatty acids which contain no (or essentially only isolated) double bonds produced no 2-propenal. However, when fatty acids contain two or more methylene-interrupted double bonds, the amount of the enal increases (nonlinearly) with increased unsaturation. Note that linoleic acid in trilinolein has one set of methylene-interrupted double bonds and produced 4.2% 2-propenal, whereas menhaden oil, which has other fatty acids that contain 4, 5 and 6 double bonds in addition to linoleic and linolenic acids, produced 31.2% 2-propenal.

The quantity of the 2-alkenal observed from the six samples prompted us to evaluate this aldehyde for its room-odor contributions. An odor panel evaluation indicated that acrolein, as a single component in a bland, heated cottonseed oil, produced only a harsh and sharp room odor.

In retrospect, heated trilinolenin produced 23 primary volatiles, which accounted for 87% of the total integrated GC area. Some of the aldehydes and other classes of compounds are anticipated from linolenate's monohydroperoxides. Either the precursors of decatrienal, nonadienal and hexenal or the compounds themselves undergo reactions that prevent the three aldehydes, accumulation. The formation of furans and 4,5-epoxy-2-heptenal suggests that more than one important oxidation/decomposition reaction takes place. The quantity of acrolein formed apparently is related to the number and location of double bonds in a heated fatty acid.

## ACKNOWLEDGMENT

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# New Sources of γ -Linolenic Acid

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## ABSTRACT

γ-Linolenic acid ($18{:}3^{\Delta 6,9,12}$) occurs in significant amounts in various species of plants surveyed. Of the species analyzed in this study, *Nonnea macrosperma*, with 5.1% γ-linolenic acid in the seed, is the richest source of this fatty acid. Other species in the same family (Boraginaceae) are also good sources: *Adelocaryum coelestinum*, *Alkanna froedinii*, *Alkanna orientalis* and *Brunnera orientalis*. *Scrophularia marilandica* (family Scrophulariaceae) seeds contain 37.9% oil, of which 9.6% is γ-linolenic acid. All species mentioned above are better sources, when the total amount of γ-linolenic acid in the seed is considered, than that used traditionally, Evening Primrose (*Oenothera biennis*, family Onagraceae). None of the other Onagraceae nor any of the *Ribes* (family Saxifragaceae) species analyzed are as rich in γ-linolenic acid as Evening Primrose. Octadecatetraenoic acid ($18{:}4^{\Delta 6,9,12,15}$) was found in significant amounts in most of the Boraginaceae and *Ribes* surveyed. The Onagraceae and Scrophulariaceae lack detectable amounts of this fatty acid.

## INTRODUCTION

Recently, there has been much interest in γ-linolenic (all *cis*-6,9,12-octadecatrienoic) acid due to claims of its involvement in preventing or alleviating a wide variety of human diseases (1-4). The most common source of this fatty acid is Evening Primrose (*Oenothera biennis*) seed oil; however, several species of the Boraginaceae, Aceraceae and Ranunculaceae families are also known to contain significant amounts (5-9). In our search for new oilseed crops we have found additional sources of this fatty acid in 45 different species.

## EXPERIMENTAL PROCEDURES

Oil content of the seeds analyzed was determined by Butt extraction (10) with petroleum ether as solvent, and is based on the dry weight unless otherwise noted. Oil was transesterified with 10% $BF_3$ in MeOH according to the method of Metcalfe et al. (11), and analyzed in a Hewlett Packard 7610 gas chromatograph using two columns simultaneously: a 10 ft × ¼ in glass column packed with 5% LAC-2-R 446 on Gas-Chrom Q, and a 4 ft × ¼ in glass column packed with 5% Apiezon L on Chromosorb W AW DMCS, at 185 C. If γ-linolenic acid was previously reported in the family, as in the Boraginaceae and Onagraceae, identification was made solely on the basis of equivalent chain lengths (ECL) with known saturated fatty acid methyl esters as standards (12). For the two remaining genera, one species of each was analyzed as follows.

A triene isomer standard was prepared from fatty acid methyl esters of *Echium plantagineum* L. The $C_{18}$ tetraene ($18{:}4^{\Delta 6,9,12,15}$) methyl ester was isolated by high performance liquid chromatography (HPLC) on a Whatman Partisil M9 10/50 ODS-2 column with acetonitrile as solvent and a differential refractometer as detector. A 24-mg sample of the tetranene was then reduced with hydrazine (13) until trienes comprised ca. 40% of the total. The trienes were separated from the mixture under the same HPLC conditions as above and analyzed by gas chomatography (GC) using a 25-m SP-1000 glass capillary column, 0.25 mm id, at 180 C. Trienes with ECL of 18.95, 19.13, 19.21 and 19.29 were obtained. ECL of 18.95 and 19.29 were identified as γ- and α-linolenic acids, respectively, by comparison with standards. The other two trienes, presumably $C18{:}3^{\Delta 6,9,15}$ and $C18{:}3^{\Delta 6,12,15}$, were not identified.

Triene methyl esters from *Ribes alpinum* L. and *Scrophularia marilandica* L. were purified by HPLC as described above, and the two mixtures of α- and γ-linolenic were analyzed by glass capillary GC. Each produced two peaks with ECL of 18.93-18.95 and 19.29, representing the presumed γ-linolenic and α-linolenic. Verification of the $C_6$ double bond of γ-linolenic acid in the mixtures was made by ozonolysis and triphenylphosphine reduction (14) followed by GC-mass spectrometry (MS) (GC: 5% BDS in a 2m × 2mm glass column, programmed from 50 to 220 C at 5 C/min; MS by electron impact in a Kratos MS-30 mass spectrometer).

## RESULTS AND DISCUSSION

### Location of the Double Bonds

The C9 and C6 aldehyde esters of the ozonolysis products from the two *Ribes* and *Scrophularia* linolenic mixtures