

# Synthesis and Occurrence of Oxoaldehydes in Used Frying Oils

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As part of our efforts to identify volatile decomposition products in used frying oils, a series of 4- and 5-oxoaldehydes were synthesized, purified, and characterized by gas chromatography, gas chromatography–mass spectrometry, gas chromatography–Fourier transform infrared spectrometry, and nuclear magnetic resonance spectrometry. Oxoaldehydes have been proposed as possible precursors of alkylfurans, which have potential anticancer effects. In a model reaction 4-oxononanal was refluxed in hexane for 40 days and only trace amounts of 2-pentylfuran were produced, suggesting that it is not a major precursor of the furan. The volatile constituents of used frying oils obtained from commercial food processors were studied, and 4-oxohexanal, 4-oxooctanal, 4-oxononanal, and 4-oxodecanal were identified.

**Keywords:** Oxoaldehydes; odor threshold; frying oil

## INTRODUCTION

During the course of our work on the volatile constituents of used frying oils we identified a series of alkylfurans ranging from 2-butylfuran to 2-octylfuran. 2-Heptylfuran, which induces increased activity of the detoxifying enzyme glutathione *S*-transferase, has been reported to inhibit chemically induced carcinogenesis in mice. Lam et al. (1993) found that 2-heptylfuran reduced benzo[*a*]pyrene-induced forestomach tumors and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumors in A/J mice. Given the possible importance of these compounds, we became interested in investigating their mechanism of formation. Chang et al. (1966) proposed a possible mechanism for the formation of 2-pentylfuran via 4-oxononanal from cyclization of the enol form. Neff et al. (1983) found that thermal decomposition of methyl 9-hydroperoxy-10,13-epidioxy-11-octadecenoate produced 2-pentylfuran and suggested that 4-oxo-2-nonenal may also be a precursor of 2-pentylfuran. To investigate the formation of alkylfurans, we synthesized a series of 4- and 5-oxoaldehydes. As part of our ongoing study to investigate the possible health hazards of repeatedly used frying oils, we have been studying the volatile fraction. We checked for the presence of oxoaldehydes in used frying oils, the details of which are reported in this paper.

## EXPERIMENTAL PROCEDURES

**Chemicals.** The  $\gamma$ - and  $\delta$ -lactones were obtained from Bedoukian Research Inc. (Danbury, CT). Pyridinium chlorochromate was received from Aldrich Chemical Co. (Milwaukee, WI).

**Dynamic Headspace Sampling.** Used frying oil (200 mL, obtained from a local food-processing plant; soybean oil was used to fry beef, veal, and breaded chicken) was placed in a 1-L round-bottom flask. A Pyrex glass head was attached to the top of the flask which allowed purified nitrogen to pass over the surface of the stirred oil and exit out of the top through a Tenax trap [glass tube 14 cm  $\times$  2.2 cm i.d. terminating in ball and socket joints; 10 g of Tenax (Alltech Associates, Deerfield, IL)]. Sampling was continued at 50 °C (maintained with a water bath) for 6 h at 3 L/min. The collected volatiles were eluted from the trap with freshly distilled diethyl ether containing 0.001% Ethyl antioxidant 330 [1,3,5-trimethyl-2,4,6-tris(3,5-di-*tert*-butyl-4-hydroxybenzyl)-benzene; Ethyl Corp., Baton Rouge, LA] and carefully concen-

**Table 1. Reaction Products of the Thermal Treatment of 4-Oxononanal<sup>a</sup>**

| peak no. <sup>b</sup> | product               | % area |
|-----------------------|-----------------------|--------|
| 8                     | 4-oxononanal          | 79.41  |
| 6                     | 3-octanone            | 8.17   |
| 9                     | $\gamma$ -nonalactone | 5.25   |
| 2                     | 2-hexanone            | 1.56   |
| 5                     | 2-hexanol             | 0.95   |
| 3                     | 1-methylcyclopentanol | 0.78   |
| 4                     | 3-hexanol             | 0.74   |
| 1                     | 3-hexanone            | 0.48   |
| 7                     | 2-pentylfuran         | 0.18   |

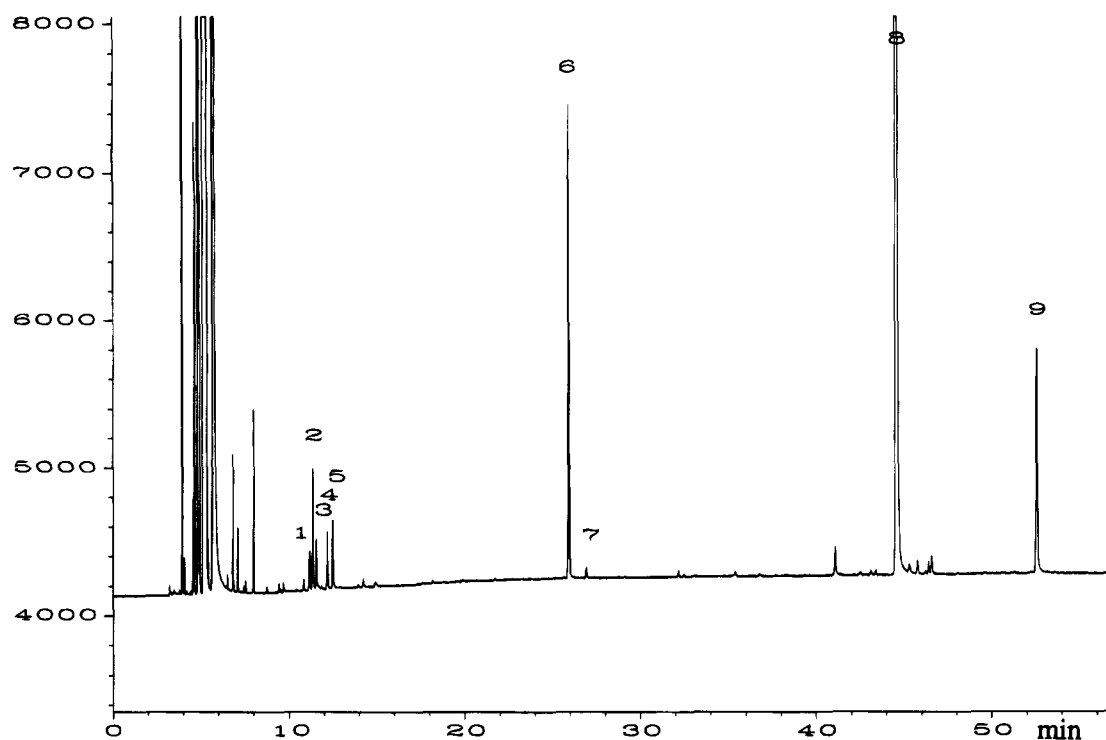
<sup>a</sup> Starting material was 95.80% pure; the remaining 4.20% was  $\gamma$ -nonalactone. <sup>b</sup> Peak numbers correspond to those in Figure 1.

trated with a Vigreux column to a final volume of ca. 100  $\mu$ L. Five headspace extracts were combined and fractionated by column chromatography.

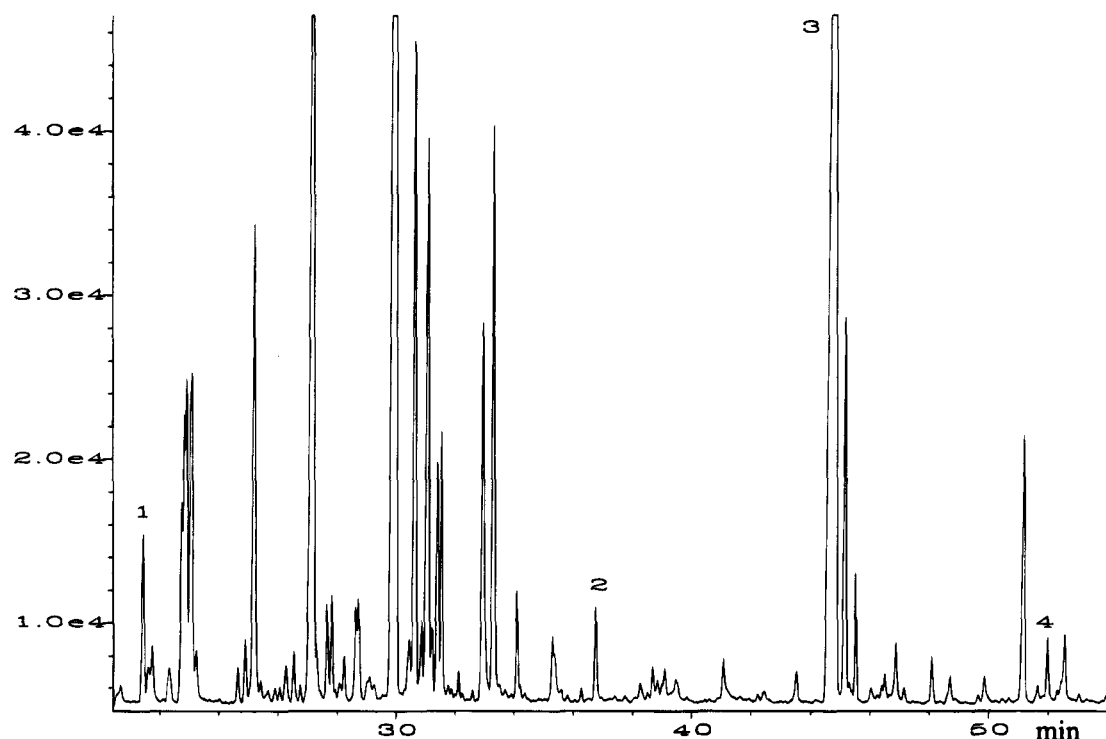
**Simultaneous Steam Distillation–Extraction (SDE).** Three different samples of used frying oil were examined. Used frying oil (250 mL) was placed in a 2-L round-bottom flask along with 250 mL of Milli-Q water (Millipore, Bedford, MA). The mixture was subjected to SDE at atmospheric pressure for 2 h with 100 mL of pentane–diethyl ether (1:1 v/v) using the SDE head described by Schultz et al. (1977). The extract was dried overnight with anhydrous sodium sulfate and concentrated with a Vigreux column to a final volume of 0.3–0.4 mL. Three batches of used frying oil (total volume, 750 mL) were sampled in this manner. The extracts were combined and fractionated by column chromatography.

**Preseparation by Adsorption Chromatography.** The combined extracts were fractionated on a silica gel column [220 mm  $\times$  22 mm (i.d.); 230–400 mesh, grade 60, Merck]. For samples prepared by dynamic headspace sampling the following stepwise elution was performed: 100 mL of pentane (fraction 1), 100 mL of 5:95 diethyl ether–pentane (v/v; fraction 2), 100 mL of 10:90 diethyl ether–pentane (v/v; fraction 3), 100 mL of 20:80 diethyl ether–pentane (v/v; fraction 4), 100 mL of 40:60 diethyl ether–pentane (v/v; fraction 5), and 100 mL of diethyl ether (fraction 6). For samples obtained by SDE the following stepwise elution was performed: 100 mL of pentane (fraction A), 100 mL of 10:90 diethyl ether–pentane (v/v; fraction B), 100 mL of 20:80 diethyl ether–pentane (v/v; fraction C), 100 mL of 40:60 diethyl ether–pentane (v/v; fraction D), 100 mL of 60:40 diethyl ether–pentane (v/v; fraction E), and 100 mL of diethyl ether (fraction F).

**Preparative Gas Chromatography.** A Carbowax 20M column [2.5 m  $\times$  6 mm i.d. glass column packed with Carbowax 20M (1% w/w) on Chromosorb G, 120–140 mesh] was em-



**Figure 1.** Gas chromatogram of products formed from the thermal treatment of 4-oxononanal. Temperature was programmed from 30 (4 min isothermal) to 200 °C at 2 °C/min on a 60 m  $\times$  0.32 mm (i.d.) DB-1 column. The peak numbers correspond to the numbers in Table 1.



**Figure 2.** Gas chromatogram of fraction E separated on a 60 m  $\times$  0.32 mm (i.d.) DB-1 column. Fraction E was obtained by separation of used oil volatiles by silica gel column chromatography. Peaks 1, 2, 3, and 4 represent 4-oxohexanal, 4-oxooctanal, 4-oxononanal, and 4-oxodecanal, respectively.

ployed. 5-Oxooctanal and 4-oxodecanal were purified on a 4 m  $\times$  6 mm i.d. glass column packed with 1% (w/w) SF-96 on Chromosorb G (80–100 mesh). The GC column effluent was split in a ratio of 1:10 to an FID and heated external port (for trapping solutes). Samples were trapped in glass capillary tubes cooled with dry ice. The oven temperature was programmed from 105 to 170 °C at 2 °C/min; the starting temperature for 4-oxodecanal was 120 °C.

**Capillary Gas Chromatography.** A HP 5890 gas chromatograph equipped with a flame ionization detector (FID)

was used. A DB-1 fused silica capillary column (60 m  $\times$  0.32 mm i.d.;  $d_f$  = 0.25  $\mu$ m; J&W Scientific, Folsom, CA) was employed. Split injection was used (1:24). The oven temperature was programmed from 30 (4 min isothermal) to 200 °C at 2 °C/min. The injector and detector temperatures were 190 and 300 °C, respectively.

**Capillary Gas Chromatography–Mass Spectrometry (GC–MS).** A HP 5890 gas chromatograph equipped with a split/splitless injector was coupled to a HP 5970B mass selective detector (capillary direct interface). A 60 m  $\times$  0.32

mm i.d. ( $d_f = 0.25 \mu\text{m}$ ) DB-1 fused silica capillary column was used with the following temperature program:  $30^\circ\text{C}$  (4 min isothermal) to  $200^\circ\text{C}$  at  $2^\circ\text{C}/\text{min}$ . Split injection was employed (1:22). The injector temperature was  $190^\circ\text{C}$ , and the transfer line temperature was  $210^\circ\text{C}$ .

**Capillary Gas Chromatography–Fourier Transform Infrared Spectroscopy (GC–FTIR).** A HP 5965B infrared detector was interfaced with a HP 5890 Series II gas chromatograph and controlled by a HP G1034A MS/IR ChemStation. A DB-1 fused silica capillary column ( $25 \text{ m} \times 0.32 \text{ mm}$  i.d.;  $d_f = 0.52 \mu\text{m}$ ) was employed. Splitless injection was used (purge delay time was 45 s). Light pipe and transfer line temperatures were  $200$  and  $210^\circ\text{C}$ , respectively. Vapor phase spectra were recorded from  $550$  to  $4000 \text{ cm}^{-1}$  with a resolution of  $8 \text{ cm}^{-1}$ .

**Synthesis and Spectral Data of Reference Oxaldehydes.** **4-Oxohexanal.** 1,4-Hexanediol, prepared by the reduction of  $\gamma$ -hexalactone with  $\text{LiAlH}_4$ , was oxidized with pyridinium chlorochromate (Corey and Suggs, 1975): FTIR (vapor phase,  $\nu$ ,  $\text{cm}^{-1}$ ) 2984, 2916, 2817, 2720, 1735, 1417, 1359, 1116, 1020, 969, 860;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.08 (3H, t,  $J = 7.3 \text{ Hz}$ , H-6), 2.51 (2H, q,  $J = 7.3 \text{ Hz}$ , H-5), 2.76 (4H, m, H-3, H-2), 9.71 [1H, t (not resolved), H-1]; MS ( $m/z$ , %) 114 ( $\text{M}^+$ , <1), 86 (53), 85 (60), 72 (5), 57 (100), 55 (9), 43 (9), 42 (9); Kovats index (DB-1) 909.

**4-Oxoheptanal.** 1,4-Heptanediol, prepared by the reduction of  $\gamma$ -heptalactone with  $\text{LiAlH}_4$ , was oxidized with pyridinium chlorochromate (Corey and Suggs, 1975): FTIR (vapor phase,  $\nu$ ,  $\text{cm}^{-1}$ ) 2971, 2916, 2817, 2720, 1733, 1415, 1364, 1128, 1017;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.92 (3H, t,  $J = 7.4 \text{ Hz}$ , H-7), 1.63 (2H, m, H-6), 2.46 (2H, t,  $J = 7.3 \text{ Hz}$ , H-5), 2.74 (4H, m, H-3, H-2), 9.81 (1H, t,  $J = 0.5 \text{ Hz}$ , H-1); MS ( $m/z$ , %) 128 ( $\text{M}^+$ , <1), 110 (6), 100 (26), 85 (67), 72 (24), 71 (63), 57 (29), 55 (10), 43 (100), 41 (41); Kovats index (DB-1) 998.

**4-Oxooctanal.** 1,4-Octanediol, prepared by the reduction of  $\gamma$ -octalactone, was oxidized with pyridinium chlorochromate (Corey and Suggs, 1975): FTIR (vapor phase,  $\nu$ ,  $\text{cm}^{-1}$ ) 2967, 2943, 2818, 2720, 1732, 1415, 1362, 1128, 1086, 1031, 864;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.91 (3H, t,  $J = 7.3 \text{ Hz}$ , H-8), 1.32 (2H, m, H-7), 1.58 (2H, m, H-6), 2.47 (2H, t,  $J = 7.4 \text{ Hz}$ , H-5), 2.75 (4H, m, H-3, H-2), 9.81 (1H, t,  $J = 0.5 \text{ Hz}$ , H-1); MS ( $m/z$ , %) 142 ( $\text{M}^+$ , 1), 124 (5), 114 (7), 100 (23), 85 (100), 72 (43), 58 (15), 57 (89), 55 (12), 43 (29), 41 (45); Kovats index (DB-1) 1099.

**5-Oxooctanal.** 1,5-Octanediol, prepared by the reduction of  $\delta$ -octalactone with  $\text{LiAlH}_4$ , was oxidized with pyridinium chlorochromate (Corey and Suggs, 1975): FTIR (vapor phase,  $\nu$ ,  $\text{cm}^{-1}$ ) 2968, 2906, 2813, 2714, 1735, 1420, 1365, 1298, 1128, 1031;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.91 (3H, t,  $J = 7.4 \text{ Hz}$ , H-8), 1.60 (2H, m, H-7), 1.90 (2H, quintet,  $J = 7.1 \text{ Hz}$ , H-3), 2.37 (2H, t,  $J = 7.3 \text{ Hz}$ , H-6), 2.48 (4H, m, H-4, H-2), 9.76 (1H, t,  $J = 1.4 \text{ Hz}$ , H-1); MS ( $m/z$ , %) 124 (2), 114 (26), 99 (21), 86 (9), 71 (100), 58 (11), 55 (23), 43 (92), 41 (49); Kovats index (DB-1) 1110.

**4-Oxononanal.** 1,4-Nonanediol, prepared by the reduction of  $\gamma$ -nonalactone with  $\text{LiAlH}_4$ , was oxidized with pyridinium chlorochromate (Corey and Suggs, 1975): FTIR (vapor phase,  $\nu$ ,  $\text{cm}^{-1}$ ) 2965, 2938, 2818, 2720, 1731, 1415, 1363, 1220, 1128, 1088, 1040, 865;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.89 (3H, t,  $J = 7.1 \text{ Hz}$ , H-9), 1.29 (4H, m, H-8, H-7), 1.60 (2H, m, H-6), 2.46 (2H, t,  $J = 7.5 \text{ Hz}$ , H-5), 2.74 (4H, m, H-3, H-2), 9.81 (1H, t,  $J = 0.5 \text{ Hz}$ , H-1); Kovats index (DB-1) 1200.

**5-Oxononanal.** 1,5-Nonanediol, prepared by the reduction of  $\delta$ -nonalactone with  $\text{LiAlH}_4$ , was oxidized with pyridinium chlorochromate (Corey and Suggs, 1975): FTIR (vapor phase,  $\nu$ ,  $\text{cm}^{-1}$ ) 2964, 2813, 2714, 1734, 1452, 1420, 1364, 1128, 1038;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  0.90 (3H, t,  $J = 7.3 \text{ Hz}$ , H-9), 1.30 (2H, m, H-8), 1.55 (2H, m, H-7), 1.90 (2H, quintet,  $J = 7.1 \text{ Hz}$ , H-3), 2.39 (2H, t,  $J = 7.3 \text{ Hz}$ , H-6), 2.48 (4H, m, H-4, H-2), 9.76 (1H, t,  $J = 1.4 \text{ Hz}$ , H-1); MS ( $m/z$ , %) 138 (2), 128 (4), 114 (29), 99 (27), 86 (31), 85 (70), 71 (84), 58 (47), 57 (100), 55 (45), 43 (45), 41 (83); Kovats index (DB-1) 1212.

**4-Oxodecanal.** 1,4-Decanediol, prepared by the reduction of  $\gamma$ -decalactone with  $\text{LiAlH}_4$ , was oxidized with pyridinium chlorochromate (Corey and Suggs, 1975): FTIR (vapor phase,  $\nu$ ,  $\text{cm}^{-1}$ ) 2935, 2872, 2818, 2720, 1730, 1460, 1416, 1363, 1278, 1217, 1128, 1090, 1044, 866, 729;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )

$\delta$  0.89 (3H, t,  $J = 7.3 \text{ Hz}$ , H-10), 1.29 (6H, m, H-9, H-8, H-7), 1.60 (2H, m, H-6), 2.46 (2H, t,  $J = 7.1 \text{ Hz}$ , H-5), 2.74 (4H, m, H-3, H-2), 9.81 (1H, t,  $J = 0.5 \text{ Hz}$ , H-1); MS ( $m/z$ , %) 170 ( $\text{M}^+$ , 1), 152 (1), 142 (4), 128 (6), 124 (4), 113 (45), 100 (30), 85 (96), 72 (58), 71 (13), 58 (15), 57 (53), 55 (26), 43 (100), 41 (47); Kovats index (DB-1) 1303.

**Nuclear Magnetic Resonance (NMR) Spectroscopy.** NMR spectra were taken on a Bruker ARX 400 spectrometer (400 MHz).

**Determination of Polar Compounds in Frying Oils.** This was done according to AOCS Official Method Cd 20-91 (revised 1993; reapproved 1993).

**Odor Threshold Determinations.** Thresholds were determined on purified samples (preparative gas chromatography) as described previously (Guadagni and Buttery, 1978) using a panel of 16–22 members. Solutions were presented in odor free Teflon squeeze bottles equipped with Teflon tubing at the top.

**Model Reaction Studies of 4-Oxononanal.** 4-Oxononanal (2 mg, 95.80%), 1 drop of glacial acetic acid, and 20 mL of hexane were refluxed with constant stirring for 40 days. The mixture was initially sampled (2  $\mu\text{L}$ ) and analyzed by gas chromatography every 2 h during the first 12 h of the reaction and daily thereafter. After 40 days, the mixture was concentrated to approximately 1 mL and subjected to GC and GC–MS.

## RESULTS AND DISCUSSION

A series of 4- and 5-oxoaldehydes were synthesized, purified by preparative gas chromatography, and characterized by NMR, GC–FTIR, and GC–MS. 4-Oxononanal has been proposed as a possible precursor of 2-pentylfuran (Chang et al., 1966). To test this theory, 4-oxononanal (95.80% pure, the remaining 4.20% was  $\gamma$ -nonalactone) was refluxed in hexane with a small amount of acetic acid for 40 days. Figure 1 shows the gas chromatogram of products formed in the thermal treatment of 4-oxononanal. Their quantitative distribution is shown in Table 1. The majority of the starting material was unchanged. The major reaction product was 3-octanone. A very small amount of 2-pentylfuran was formed. Though the reaction of 4-oxononanal may be different under frying conditions, it does not appear to be a major source of 2-pentylfuran. However, this postulate should be tested under other reaction conditions, i.e. adding isotopically labeled 4-oxononanal to oil and heating under typical frying conditions.

Volatile constituents of used frying oil were isolated by dynamic headspace sampling and atmospheric SDE. Sample constituents were identified by comparison of the compound's Kovats index ( $I$ ) and mass spectrum with those of a reference standard. 4-Oxoaldehydes were identified in both the headspace and SDE samples. The headspace volatiles were separated on a silica gel column into six fractions (1–6). 4-Oxohexanal, 4-oxooctanal, and 4-oxononanal were identified in fraction 6. They occurred at 0.07, 0.02, and 0.26%, respectively, of the total headspace volatiles as measured in the unfractionated headspace sample. The SDE volatiles were separated on a silica gel column into six fractions (A–F). 4-Oxohexanal, 4-oxooctanal, 4-oxononanal, and 4-oxodecanal were identified in fraction E (Figure 2). They constituted 0.55, 0.31, 9.12, and 0.21%, respectively, of the total volatiles in fraction E. Additionally, 4-oxononanal also was present as 0.53% of the total volatiles in fraction D. Two additional frying oil samples (prepared by SDE and fractionated by silica gel chromatography as above) were analyzed for oxoaldehydes. Sample 2 (soybean oil; content of polar compounds was 12.0%) contained 4-oxohexanal, 4-oxooctanal, 4-oxonona-

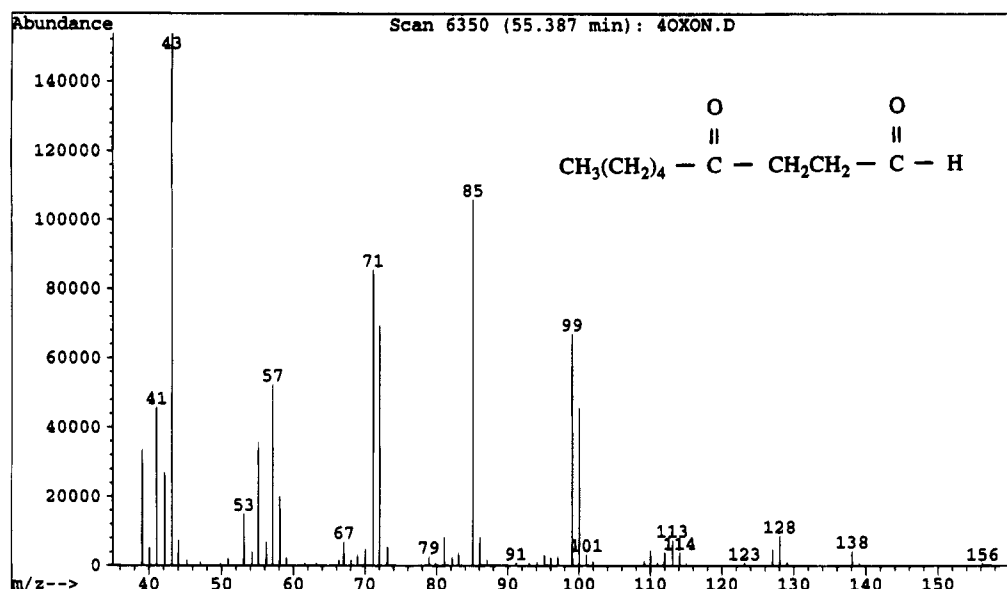


Figure 3. Mass spectrum of 4-oxononanal.

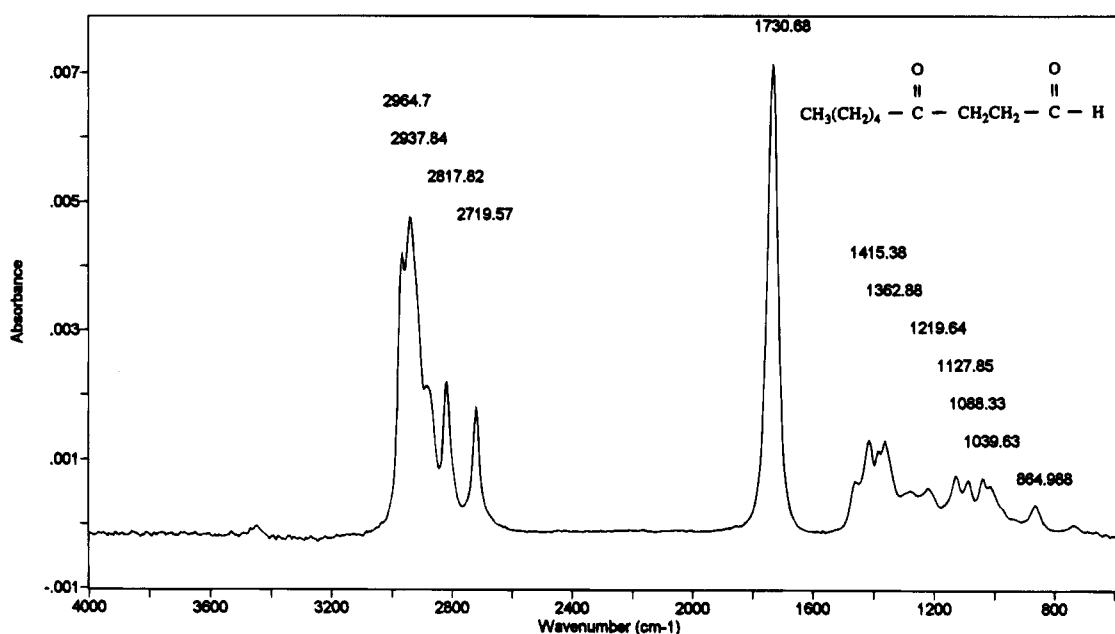


Figure 4. Vapor phase FTIR spectrum of 4-oxononanal.

nal, and 4-oxodecanal at 0.57, 0.46, 15.19, and 0.26%, respectively, of the total volatiles of fraction E. Sample 3 (canola oil; content of polar compounds was 8.8%) contained 4-oxohexanal and 4-oxononanal at 0.05 and 2.86%, respectively, of the total volatiles of fraction E. The mass spectrum and FTIR data for 4-oxononanal are shown in Figures 3 and 4, respectively. The bands at 2818 and 2720  $\text{cm}^{-1}$  can be attributed to Fermi resonance between the fundamental aldehydic C-H stretch and the first overtone of the aldehydic C-H bending vibration (Nyquist, 1984). The C=O stretch was observed at 1731  $\text{cm}^{-1}$  which is higher than the 1720 and 1710  $\text{cm}^{-1}$  observed for this compound in the condensed phase (Yajima et al., 1985). Oxoaldehydes have not been widely reported in natural products. 2-Oxoaldehydes ( $\text{C}_2$ – $\text{C}_8$ ) were identified (as DNP-osazones) in oxidized methyl linoleate by Cobb and Day (1965). 4-Oxononanal was tentatively identified as a thermal decomposition product of methyl 9-hydroperoxy-10,13-epidioxy-11-octadecenoate by Neff et al. (1983). This oxoaldehyde was first identified as a naturally occurring

Table 2. Odor Thresholds of Some 4-Oxoaldehydes

| compd         | odor threshold (ppb, in water) | compd        | odor threshold (ppb, in water) |
|---------------|--------------------------------|--------------|--------------------------------|
| hexanal       | 5                              | nonanal      | 1                              |
| 4-oxohexanal  | 3000                           | 4-oxononanal | 150                            |
| heptanal      | 3                              | decanal      | 2                              |
| 4-oxoheptanal | 800                            | 4-oxodecanal | 12                             |

flavor constituent by Yajima et al. (1985), who found it in watermelon. It has also been tentatively identified as a constituent of roasted chicken fat (Noleau and Toulemonde, 1987). Spectral data for various dicarbonyl compounds including 3-oxo- and 5-oxodecanal were reported by Guth and Grosch (1989).

The odor threshold values of some of the 4-oxoaldehydes were determined using sensory panel methods. The data listed in Table 2 show that the addition of a keto group in the 4-position causes an increase in the odor threshold but that the effect becomes smaller as the chain length increases. In comparison, Guth and

Grosch (1989) found the related 3-oxodecanal and 5-oxodecanal had odor thresholds of 0.35–0.70 and 1.1–2.2 ng/L air, respectively. 4-Oxononanal has been patented for use in perfume compositions (Hasegawa, 1981). The three frying oils examined in this study contained approximately 1–10 ppm of 4-oxononanal. At this level 4-oxononanal may contribute to the odor of used frying oil. The toxicological importance of these oxoaldehydes is unknown and will be the subject of future investigations.

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