Volatile Compounds Produced During Deodorization of Soybean Oil and Their Flavor Significance

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ABSTRACT: Freshly deodorized soybean oil has a characteristic nutty flavor but often yields no detectable headspace volatiles. The cause of this flavor was investigated by deodorizing soybean oil in an apparatus with a double cold trap that allowed the volatile compounds formed from the initial decomposition of hydroperoxides to be collected separately from those produced during the normal deodorization process. The chief volatile components from the normal deodorization process were hydrocarbons, which contributed little to no odor to the oil. The compounds with the greatest odor were carbonyls, especially heptanal and *cis*-4-heptenal. Although these components should accumulate at some steady-state concentration in an oil during its deodorization, none seemed to account for the flavor of the deodorized oil. By using a particle detector, it was shown that small particles could be generated in the human mouth that could provide a mechanism to bring oil with nonvolatile flavor components into contact with the olfactory organ. Attempts to separate possible nonvolatile flavors in deodorized oil from triacylglycerides by chromatography on alumina or reaction with 2,4-dinitrophenylhydrazine were unsuccessful. Possibly, the flavor is caused by the glycerol esters themselves.

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KEY WORDS: Carbonyl, deodorization, flavor, GC–MS identification, hydrocarbon, sensory evaluation, soybean oil, volatile compounds.

Freshly deodorized soybean oils have a nutty flavor that is considered to be acceptable by consumers. Sensory panel members, looking for flavors caused by oxidation in soybean oils, learn to discount this nutty flavor, but when the flavor intensity of freshly deodorized oils is compared with a standard, such as an odorless mineral oil, the freshly deodorized oil receives a flavor intensity that cannot be accounted for by recognized flavor volatiles that arise from oxidation (1). Nonfat or reduced-fat foods often lack desirable flavors associated with fresh vegetable oils, such as soybean oils, and this observation may be partly related to the nutty flavor. It is likely that volatile compounds do not exist in large amounts in freshly deodorized oil because of the high-vacuum and temperature treatments; therefore, little work has been done to examine the flavor compounds in freshly deodorized vegetable oil.

In this study, we wished to see if we could account for the flavor of freshly deodorized oil by examining the volatile flavor compounds generated during deodorization and by exploring the possible role of nonvolatile flavor compounds.

EXPERIMENTAL PROCEDURES

Collection of volatiles. Newly purchased Wesson brand soybean oil (800 g) was deodorized with steam at 235°C under vacuum (0.005 mm Hg) as described by Stone and Hammond (2) and modified by Moulton (3). The volatiles that formed were collected in one cold trap (Fig. 1) for 2 h, and then the apparatus was switched to a second cold trap for 4 h.

Separation of volatiles from trap 2 into carbonyls and hydrocarbons. The volatiles in trap 2 were extracted three times with 10 mL pentane, and the pentane extract was concentrated to 1.5 mL. To retain the low-boiling volatile compounds, the pentane extract was placed in a 10-mL tube sitting in a water bath, which was set at 2°C above the boiling point of pentane. The solvent was slowly evaporated while keeping the level of pentane higher than the surrounding water. Florisil (magnesium silicate, 60–100 mesh; Sigma Chemical Company, St. Louis, MO) was activated at 250°C overnight, and 10% of its weight of distilled water was added and allowed to equilibrate for 1 d. Next, 700 µL of the pentane concentrate was applied

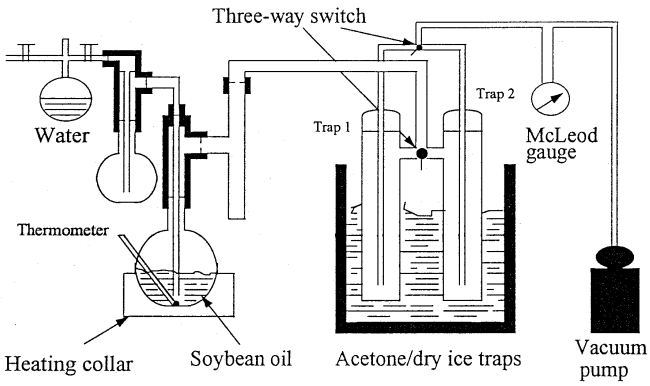

FIG. 1. Diagram of deodorization apparatus.

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to a 10-mm i.d. × 26-cm column filled with 10 g Florisil in pentane/ether (99:1). Hydrocarbons were eluted with 15 mL pentane/ether (99:1), and the carbonyls were eluted with 15 mL ether (4).

Instrumental analysis and gas chromatography (GC) odor evaluation. The volatiles were analyzed by GC (HP 5890 series II; Hewlett-Packard, Palo Alto, CA) on a Supelco SPB-1 fused-silica capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness; Bellefonte, PA) at a helium flow rate of 1.7 mL/min. The temperature program was held for 4 min at 30°C, raised from 30 to 210°C at 6°C/min, and finally held at 210°C for 10 min. Peaks were detected by a flame-ionization detector (FID) held at 230°C. Volatiles were identified by mass spectrometry (MS) (HP 5970 series Mass Selective Detector), and when possible, by comparison of their retention times with those of known standards. Odor evaluations were accomplished by three trained, experienced sensory panelists, who smelled individual volatiles as they issued from the GC detector port. For peaks with detectable odor, the intensity and sensory descriptors were recorded. The GC temperature program for odor evaluation was the same as that used for GC analysis. The sniffing port was adapted at the elution end of the GC column by blowing out the flame of the FID. No additional attachments were added.

Measurement of aerosol particles generated in human mouths. Particles in the 0.5 to 5 μm diameter range were measured with a Met One particle detector (Met One Inc., Grants Pass, OR). The counter was placed in a biological safety cabinet (Nuaire, Plymouth, MN), and panelists breathed particle-free air from inside the cabinet until their breath revealed no particles (~1 min). Then, the panelists generated particles by smacking their lips or pulling their tongues away from the roofs of their mouths. The number of particles so generated were counted by inserting the inlet hose of the counter near or between the lips and sampling at a flow of 2.8 L/min.

Sensory evaluation of soybean oil before and after alumina fractionation. Wesson brand soybean oil (200 g) was deodorized at 220°C for 6 h under vacuum (0.005 mm Hg), and 100 g was immediately fractionated by passage through a 35-mm i.d. × 57-cm column filled with 200 g alumina in hexane (5). Triacylglycerides (TAG) and the compounds less polar than TAG were eluted with 2 L of hexane/ether (9:1). A yield of 62 g of TAG resulted after rotary evaporation of the solvent at 45°C and steam distillation at 120°C under vacuum (0.005 mm Hg) for 1 h. The TAG, with and without alumina fractionation, were diluted 100-fold with water to make emulsions (6), and their flavors were evaluated by 10 panelists in a triangle test on two separate occasions. The samples (60 mL) were presented to the panelists in 266-mL plastic cups, covered with aluminum foil, and tasted at room temperature. Significance was accepted at $P \leq 0.05$ (7).

Synthesis of methyl 9-oxononanoate. Methyl 9-oxononanoate was prepared by the procedure of Hammond *et al*. (8) for identifying positional isomers of unsaturated fatty acids, except the carbon disulfide was replaced by pentane. A solution of 25 mg methyl oleate in 3 mL pentane was cooled in an acetone dry-ice bath, and ozone was passed through it at 20 mL/min. The presence of an excess of ozone was detected by passing the gas leaving the reaction vessel into a solution of 5% potassium iodide and a starch indicator in 5% sulfuric acid, which turned blue in the presence of ozone. When ozone absorption was complete, an excess of triphenylphosphine (100 mg) was added, and the reaction mixture was allowed to warm to room temperature. The methyl 9-oxononanoate in the ending reaction mixture (1 mL) was purified by chromatography on a 10-mm i.d. × 26-cm column filled with Florisil in hexane. Hexane/ether (9:1, 100 mL) was used to remove triphenylphosphine, and then ether was used to elute methyl 9-oxononanoate. The first 15 mL of elution was discarded, and the next 10 mL was collected. The ether fraction containing methyl 9-oxononanoate was concentrated and rechromatographed on Florisil. The refractionated methyl 9-oxononanoate ether solution was concentrated. The odor of methyl 9-oxononanoate was evaluated by two of the three trained panelists noted earlier, by smelling the GC detector port. Also, after evaporating the ether, mineral oil was added to make up 1 and 10 ppm methyl 9-oxononanoate in mineral oil, and the flavor intensity and character (presence of typical nutty flavor) were compared with a blank mineral oil by the same two panelists.

Preparation of carbonyl-free soybean oil. A 2,4-dinitrophenylhydrazine (DNPH) column was prepared according to the procedure of Schwartz and Parks (9). The DNPH (Aldrich Chemical Company Inc., Milwaukee, WI) (0.5 g) was dissolved in 6 mL 85% phosphoric acid (Fisher Scientific, Pittsburgh, PA) by grinding in a mortar, and 4 mL distilled water was added to form a yellow solution. Celite (10 g) was added to the solution and ground to make a homogeneous mixture. Then the DNPH-impregnated Celite was tamped in a 16-mm i.d. × 61-cm column filled with hexane.

Commercial soybean oil (Wesson, 50 g) was dissolved in 60 mL hexane and applied to a DNPH column. A yellowish oil was eluted with hexane (100 mL) and then passed through a column (35-mm i.d. × 57-cm) filled with 100 g alumina in hexane. The eluate (100 mL) obtained from the alumina column was passed through a new 100-g alumina column again to obtain a carbonyl- and DNPH-free, colorless soybean oil in hexane. The hexane was removed by rotary evaporation at 45°C and steam deodorization at room temperature under vacuum, and the flavor intensity and sensory description of the carbonyl-free soybean oil were compared with freshly deodorized soybean oil twice by the three trained panelists, who tasted the oil directly.

Synthesis of glyceroltricaprate (GTC). Capric acid (178 g, Sigma) and glycerol (30 g, Sigma) were heated and refluxed with 66 mL high-performance liquid chromatography-grade benzene (Fisher Scientific) and 3.13 g *p*-toluenesulfonic acid (Sigma) in a flask equipped with a Dean-Stark trap and condenser (10). The reaction was refluxed for 20 h, and 17.3 mL of water was formed and trapped in the Dean-Stark trap. Distilled water (300 mL) was added to the reaction mixture, and the organic phase (upper layer) was separated with a separa-

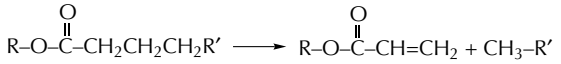
tory funnel. The organic phase (GTC and some benzene) was washed three times with 100 mL 5% sodium carbonate and three times with 100 mL distilled water. The organic phase was then dried with 23 g sodium sulfate.

Ninety-two grams of synthesized GTC (with a trace of benzene) was dissolved in 100 mL hexane and passed through a 35-mm i.d. × 57-cm column filled with 200 g alumina in hexane. The GTC was eluted with 600 mL of hexane/ether (9:1). After rotary evaporation at 80°C and steam distillation at 80°C under vacuum (0.005 mm Hg) for 1 h to remove hexane, ether, and benzene, 50 g of GTC was obtained. The GTC was then deodorized at 200°C under vacuum (0.005 mm Hg) for 1 h and purified again by passing through the 100-g alumina column. Twenty grams of GTC was obtained after rotary evaporation of solvent at 80°C and steam deodorization at 80°C under vacuum for 30 min. The GTC was evaluated for flavor intensity and description by the three trained panelists on two separate occasions.

RESULTS AND DISCUSSION

Trap contents. The double cold trap on the deodorizer was designed to capture volatiles arising from previous oxidation of the oil in trap 1 and to capture volatiles produced during deodorization in trap 2. Examination of the contents of trap 2 by GC indicated that the major components were hydrocarbons, but that carbonyl compounds accounted for most of the flavors. For better identification, the trap contents were separated into polar and nonpolar fractions by chromatography on a Florisil column.

Nonpolar fraction. The nonpolar fraction from the Florisil column was shown by GC–MS to be primarily unsaturated hydrocarbons that varied in length from 8 to 18 carbons (Fig. 2). Identification was based on the molecular weight of the largest mass peak. Compounds of less than eight carbons may have been generated but lost during evaporation of the solvent. Probably, the chains were generated by heat degradation of the carbon chains as follows:



Because 15-carbon chains were the most prevalent, seemingly scission between carbon 3 and 4 of the C_{18} chains is favored. Scission between carbon 6 and 7 or 5 and 6 also seems to be favored.

Limonene also was identified in the nonpolar fraction by its MS and retention time. It was the only compound of the nonpolar fraction to exhibit a noticeable odor when smelled at the exit port of the GC. Probably, the branched structure of limonene is generated from heat degradation of sterols or squalene rather than from fatty acyl groups.

Polar fraction. The polar fraction from the Florisil column consisted primarily of aldehydes (Fig. 3, Table 1), including heptanal, *cis*-4-heptenal, a heptanal dimer, and heptanoic acid. *Cis*-4-heptenal has been shown to arise from 2,6-nonadienal by a reversal of the aldol condensation (11), and a similar breakdown of 2-nonenal or its precursor could lead to heptanal. The carbonyls likely come from the slow decomposition of the stable dialkylperoxides formed in free-radical termination reactions. The heptanal dimer is presumed to be formed by aldol condensation of the heptanal after its release from its precursor.

Many of the other compounds of trap 2 are well-known oxidation products (1). The menadione, like limonene, may

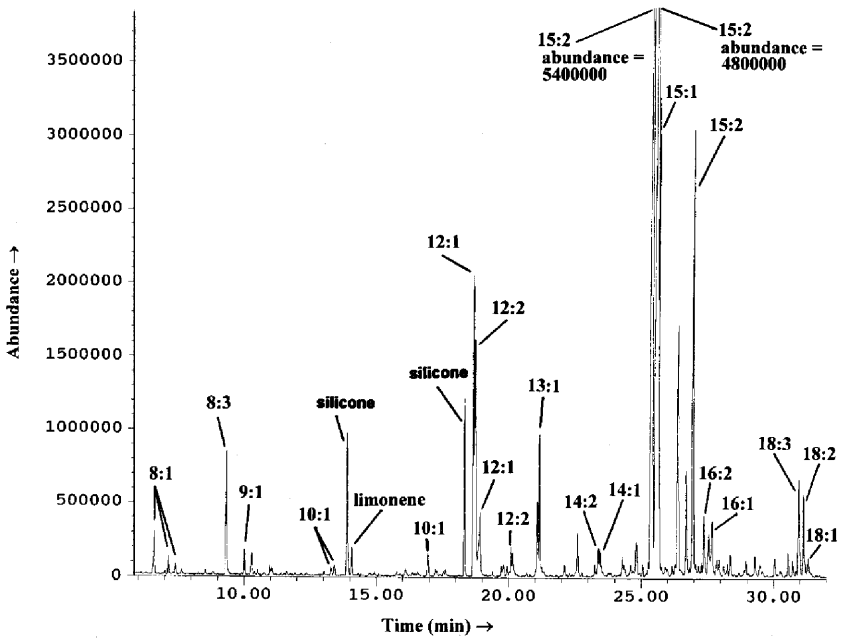


FIG. 2. Gas chromatography (GC)–mass spectrometry (MS) chromatogram of hydrocarbons formed during deodorization of soybean oil. The silicone peaks are attributed to degradation of the GC stationary phase.

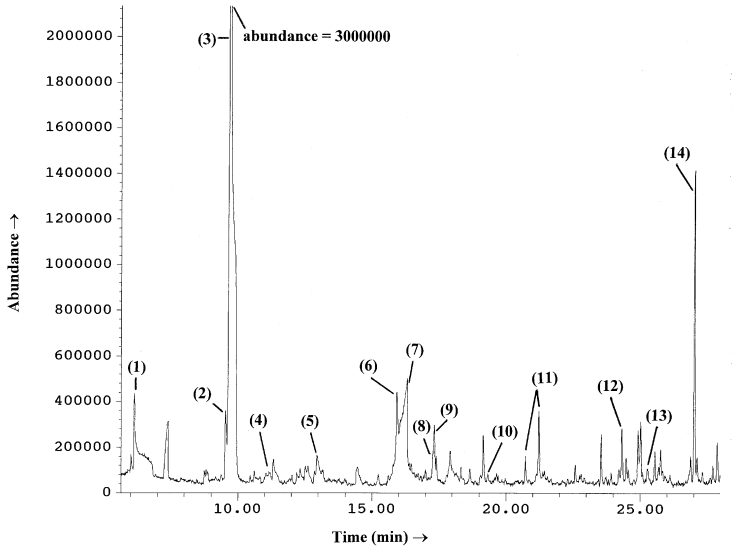


FIG. 3. GC–MS chromatogram of carbonyls formed during deodorization of soybean oil. Peak numbers are identified in Table 1. See Figure 2 for abbreviations.

come from the rearrangement of sterols or squalene or their oxidation products. 3-Methyl-2,4-nonanedione has been reported as a photoxidation product of furanoid fatty esters in soybean oil (12), but a trace seemed to be generated during deodorization. Phenylpropanone, a relatively strong fruity-rose flavor as determined by the three trained, experienced sensory panelists, may come from the degradation of linolenate. The odors of other carbonyls found in trap 2, and described by the three sensory panelists, are listed in Table 1.

The odors of the heptanal and *cis*-4-heptenal were the most potent in the polar fraction, but because they are fairly volatile, not much accumulated in the oil being deodorized, and no compounds with low volatility were present in significant amounts or had an unusually intense odor. Several peaks in Figure 3 were not further identified by MS; however, none of these had odor significance. Thus, although several volatile odor compounds were found in the deodorized oil, none seemed to be present in sufficient amounts or to have sufficient odor intensity to account for the flavor of freshly deodorized soybean oil. Previous unpublished work from our laboratory also showed that contact of freshly deodorized soybean oil with human saliva did not release volatile flavor compounds. The flavor perception of freshly deodorized oils seems to be mediated by the olfactory organ, because the nutty flavor cannot be detected if the oil is tasted with the observer's nose blocked.

Flavor from aerosol particles. An alternate mechanism that might account for the flavor of freshly deodorized oil is sug-

TABLE 1
Carbonyl Compounds Found in Trap 2

Number	Compound	Retention time (min)	Identification MS[a]	Identification RT[b]	Odor descriptor[c]
1	Hexanal	6.15	+	+	Green
2	*cis*-4-Heptenal	9.54	+	+	Fish oil
3	Heptanal	9.72	+	+	Heptanal
4	Benzaldehyde	.21	+	+	Cherry
5	2,4-Heptadienal	12.96	+	+	Fruity
6	Nonanal	15.93	+	+	Slight fruity
7	Heptanoic acid	16.3	+	+	Sweaty
8	Phenylpropanone	17.27	+	+	Fruity-rose
9	2-Nonenal	17.32	+	+	Aldehyde
10	3-Methyl-2,4-nonadione	19.3	+	+	Licorice
11	2,4-Decadienal[d]	20.73 21.22	+	+	Beany
12	Decano-γ-lactone	24.3	+	+	Buttery lactone
13	Menadione	25.28	+	+	Spicy
14	Heptanal dimer	27.01	+	—	—

[a]MS, mass spectrometry.
[b]RT, retention time.
[c]Odor identified, within the retention time of each peak, by three trained, experienced sensory panelists.
[d]*cis-cis* and *cis-trans* isomers elute at different times.

gested by the work of Hammond and Smith (13), who reported that the odor of swine houses was carried on particulates and that particulates enhanced odor intensity because they were scrubbed from air streams in the nasal passage much more efficiently than molecularly dispersed odorants. This argument is based on the observation that the olfactory organ is placed at a sharp bend in the air stream and particles in the 0.5 to 5 μM range are removed and deposited on the olfactory organ by a centrifugal effect. Thus, the load of odorants on particles of this range is released directly on the olfactory organ.

Using the three trained panelists, we found that several hundred particles in the 0.5 to 5 μM range could be generated by smacking one's lips and by pulling the tongue from the roof of one's mouth. Interestingly, these are precisely the conditions in which one can taste freshly deodorized oil. If one puts freshly deodorized oil in one's mouth and breathes in and out while keeping the lips slightly parted and tongue still, there is no flavor perception, but if one opens and closes the mouth or pulls the tongue from the roof of one's mouth while breathing gently through the mouth, the flavor is perceived.

Flavor from nonvolatile compounds. These observations encouraged us to look for nonvolatile flavor compounds in the oil. It seemed likely that the flavors would be more polar than TAG, considering the nature of the flavors found in oxidized oil. Thus, we tried an experiment that might concentrate the flavors from freshly deodorized soybean oil by passing the oil through alumina columns. No difference in flavor intensity before and after treatment was perceived in the TAG fractions eluted from such columns. This treatment would eliminate mono- and diacylglycerides or other polar compounds that might contribute to the flavor.

Aldehydes and ketones are only slightly more polar than TAG, so it seemed possible that azelaic semialdehyde esterified with glycerol, or ketoacyl groups generated by decomposition of acyl hydroperoxides esterified with glycerol could cause the flavor of freshly deodorized oils. To this end, methyl azelate semialdehyde (methyl 9-oxo-nonanoate) was synthesized by ozonolysis of methyl oleate, and its odor and flavor were examined by smelling it as it exited from the GC and tasting the compound in mineral oil solutions. Methyl 9-oxo-nonanoate had no detectable flavor or odor. Commercial soybean oil also was passed through a DNPH reaction column to convert any volatile or nonvolatile carbonyls it contained to DNPH derivatives. Then, the DNPH derivatives were removed by chromatography on alumina, and the oil was tasted after removal of the solvent at low temperature. The oil had a nutty flavor typical of freshly deodorized oils. Finally, GTC was synthesized from glycerol and capric acid. After purification on an alumina column and steam deodorization, the flavor of GTC was perceived by two panelists as nutty, whereas the other panelist identified no odor.

Thus, if there is a nonvolatile flavor component in freshly deodorized vegetable oils, it seems not to be very polar nor to be a carbonyl. The nutty flavor of GTC suggests that the nutty flavor may be caused by the glycerol esters themselves.

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