Quantitation of Flavor Volatiles in Oxidized Soybean Oil by Dynamic Headspace Analysis

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ABSTRACT: A dynamic headspace procedure was developed for isolating the volatiles from oxidized soybean oil and trapping them on an adsorbent under conditions that gave minimal decomposition of hydroperoxides (50°C for 30 min at a helium flow of 75 mL/min). The volatiles were desorbed from the adsorbent and separated by gas chromatography (GC) on a methyl silicone capillary column. Equations were derived from theoretical considerations that allowed the actual concentration of each flavor component in the oxidized oil to be calculated from the area of the GC peaks. The reliability of the method and calculations was demonstrated by recovery experiments. The concentration of 2-heptanone in a mineral oil emulsion, equivalent in flavor intensity to each component, was calculated and summed to estimate the overall flavor intensity of the samples. The overall estimations were compared with the concentrations of 2-heptanone observed to be equivalent in flavor intensity to the oxidized oil samples when these were tasted in emulsion. The concentrations of individual components calculated from the headspace volatiles data were all present at concentrations below their flavor thresholds, and the simple sum of the intensities of their flavors generally accounted for less than half of the flavor intensities of the oil samples. The differences in the headspace and sensory analyses might be attributed to the flavor of the unoxidized oil, synergistic interactions, and/or the presence of unmeasured flavors components.
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KEY WORDS: Dynamic headspace, photooxidation, sensory evaluation, soybean oil flavor, volatile quantitation.

Simple, rapid, objective methods of evaluating the oxidative flavor quality of fats and oils by nonsensory methods have long been sought. Volatile compounds, especially carbonyl compounds, generally believed to cause oxidized flavors, have been identified (1,2). Carbonyls in oxidized fats and oils have been quantitated as various derivatives (3–6), and more recently, direct gas chromatography (GC) of volatiles collected by various methods (7–12) has been used. Good correlations (r as high as 0.99) have been reported between sensory evaluation of oxidative flavor qualities and the GC volatile responses (13–16). In spite of these advances, reliable quantitation of the known flavor compounds present in oxidized fats and oils has been difficult because the peroxides formed during oxidation decompose to additional carbonyls and other volatiles during the isolation and separation procedures that have been used, and the peak areas obtained in volatile tests are biased according to the volatility of the various flavor components (17–19).

Even if reliable quantitative measures of the amounts of the flavor compounds were known, the relationship between these quantities and the intensity and character of the sensory perception that they elicit is poorly understood (20–24). Dixon and Hammond (25) proposed using dilutions of 2-heptanone in mineral oil as a standard with which the intensity of oxidized oils could be compared. They also advocated tasting such oils emulsified in water and stabilized with gum acacia to minimize carryover of one sample to the next and to improve the accuracy and repeatability of the sensory evaluations. Using this technique, they compared the flavor intensities of various concentrations of carbonyls implicated in fat oxidation against the intensity of 2-heptanone. Their attempts to compare these intensities with those of oxidized soybean oil were limited by the reliability of the quantitative analyses of carbonyls available at the time.

In this study, a technique was developed in which volatile compounds were stripped from a small amount of oil with helium at 50°C, a temperature at which decomposition of peroxides occurs slowly (26), and collected on an adsorbent. The volatile compounds were measured by GC, and their original concentrations in the oil were calculated from their retention times on nonpolar GC columns by using a formula developed from theoretical considerations. The flavor intensities of the volatile compounds were calculated in terms of the concentration of a 2-heptanone standard with the same flavor intensity. The sum of the 2-heptanone values was compared with the observed flavor intensity of the oil sample whose flavor intensity had been compared with those of standard dilutions of 2-heptanone.
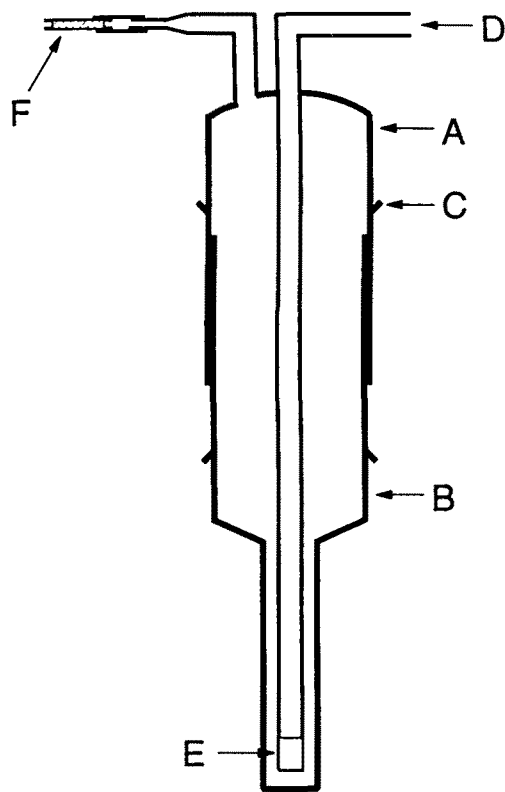
MATERIALS AND METHODS

Materials. Wesson brand soybean oil was purchased locally. Four soybean oils with various fatty acid compositions, A16,

A6, A87, and Hardin, were provided by Liu and White (27). These oils had been extracted by cold-pressing soybeans obtained from the Agronomy Department of Iowa State University (Ames, IA) and had been alkali-refined, deodorized, and stored at −18°C until used. *Cis*-3-hexenal was synthesized by oxidation of *cis*-3-hexenol according to the method of Kajiwara *et al.* (28). *Cis*-3-hexenol, flavor standards, and mineral oil for the sensory evaluation were purchased from Sigma (St. Louis, MO) and Aldrich (Milwaukee, WI).

Collection of volatiles. The apparatus used to collect the volatiles from the oil samples is shown in Figure 1. Oil (4 g) was placed in the apparatus, which was immersed in a 50°C water bath, and the flow rate of the helium that passed through the gas dispersion tube was fixed at 75 mL/min by means of a flow controller and rotometer. The volatiles stripped from the oil were trapped in a 3-mm o.d. × 72-mm glass tube filled with 43 mg of an adsorbent based on 2,6-diphenyl-*p*-phenylene oxide (Tenax TA®; Alltech Associates, Deerfield, IL), held in place by glass wool plugs. The adsorbent tubes used to collect the volatile compounds were conditioned in the inlet of a Hewlett-Packard 5890 Series II GC (Palo Alto, CA), whose inlet had been modified to receive them, for 3 h at 230°C and stored in closed containers until used. The oil sample in the apparatus was purged for 5 min to allow for equilibration of the gas flow and temperature before collection of the volatiles for 30 min.

Desorption of volatiles and GC analysis. After the absorbent tube was used in collecting volatiles, the trapped volatiles were desorbed in the GC inlet at 230°C and transferred in helium at 1.7 mL/min onto a Supelco SPB-1 fused-silica capillary column (30 m, 0.25-mm i.d., 0.25-μm film thickness; Bellefonte, PA) and condensed in the first loop of the column, which was cooled with a dry ice–acetone bath. After exactly 5 min of desorption and transfer, the dry ice–acetone bath was removed, the GC was held for 3 min at 30°C, raised from 30°C to 210°C at 6°C/min and finally held at 210°C for 5 min. Peaks were detected by a flame-ionization detector (FID) held at 220°C. Peak areas were averaged from duplicate GC runs. To determine retention times, equivalent chain numbers (ECN) and volatility constants, standards were added to fresh soybean oil or stripped soybean oil (sparged at 50°C with helium for 16 h), and the volatiles were collected and analyzed by GC as described before. Peaks were identified by comparing their retention times and mass spectra with those of known compounds. For mass spectrometry, a Hewlett-Packard 5970 mass-selective detector was used in place of the FID. To convert GC peak areas into the amounts of the various volatiles, the response factor of the FID was determined by injecting hexane solutions of known concentrations of the standards in triplicate by using a 20:1.7 injection split.

Fatty acid methyl esters. Fatty acid methyl esters, prepared according to the method of Frey and Hammond (29), were determined by a Hewlett-Packard Model 5890 GC on a J&W (Folsom, CA) DB-23 fused-silica column (15 m, 0.25-mm i.d., 0.25-mm i.d., 0.25-μm film thickness) at 200°C isothermally with an FID.

Sensory evaluation. Sensory evaluations followed the procedure of Dixon and Hammond (25) by using 1% (by weight) soybean or mineral oil emulsified in tap water with 0.65% (by weight) gum acacia. Blank emulsions were prepared from mineral oil. Emulsions prepared from mineral oil containing 2-heptanone at 0.25, 0.5, 1, 2, and 4×10^{-4} parts by weight were designated, as previously described (26), as standards A through E, respectively. Emulsions (40 mL) were presented to the panel in 266-mL plastic cups at 24°C. The emulsions were evaluated within 2 h of their preparation and were covered with aluminum foil until tasted. Data were collected from ten trained panelists who were trained for four sessions on soybean oil oxidized to various degrees. Each panelist was provided with four samples, five standards, and a blank emulsion and instructed to taste the sample emulsions in order of increasing odor intensity while comparing their intensities with those of the standards. Panelists rated the flavor intensity of samples on a 15-cm line divided into four equal lengths and labeled A through E to indicate the standard emulsions. A sample that was perceived as being weaker than A or stronger than E was rated by extending the scale line in the appropriate direction. The sensory evaluation of each oil

FIG. 1. Glass volatiles-stripping apparatus: upper (A) and lower (B) parts joined by a joint. After the oil sample is introduced, the apparatus is closed and secured by rubber bands fastened to the projections (C). Helium gas is blown into the apparatus through (D) and sparged through the dispersion tube (E), and volatiles are trapped in a tube with adsorbent (F).

sample was replicated two days after the initial evaluation. During the interval, the oil sample was stored at −18°C. For compounds not reported by Dixon and Hammond (25), the slopes and intercepts of log-log plots of concentrations giving equal flavor intensity for various flavors vs. 2-heptanone were determined by their method. The slopes and intercepts for *trans,cis*-2,4-dienals were assumed to be the same as those of the *trans,trans*-isomers because the *trans,cis*-isomers were not commercially available. The *trans,trans*-isomers contained about 10–15% *trans,cis*-isomer. Values for unknown compounds were set arbitrarily at an average value.

Storage tests. Soybean oil samples (35 g in 50-mL beakers) were oxidized in duplicate at 35°C under 1937 lux of fluorescent light and analyzed for volatiles immediately. Peroxide values (PVs) were determined according to Hamm *et al.* (30).

Statistical analysis. Statistical analyses were done by using the General Linear Model procedures of SAS 6.06 (31).

THEORY

Quantitation of carbonyl compounds. If the volatile solutes are an ideal solution in triacylglycerols, from Raoult's equation, the vapor pressure, p, is

$$p = p_0 \left[n_1/(n_1 + n_2) \right] \qquad [1]$$

where p_0 is the vapor pressure of the pure solute and n_1 and n_2 are the moles of solute and solvent, respectively. In a dilute solution such as that of a flavor volatile in oil, $n_1 <<< n_2$, and the mole fraction is approximately n_1/n_2, the molar concentration, c, of the solute.

The rate at which a volatile is sparged from the oil by a stream of gas will depend on gas flow rate, size of bubbles, efficiency of transfer, temperature, and vapor pressure of the solute. If the flow and bubble size are constant,

$$-dc/dt = k_1 p_0 c \qquad [2]$$

At a constant temperature, p_0 becomes constant and

$$-dc/dt = k_2 c \qquad [3]$$

Rearranging, we have the first-order rate equation,

$$-dc/c = k_2 t \qquad [4]$$

which can be integrated between c_0, the starting concentration of a volatile, and c, the concentration at time t, to give

$$ln(c_0/c) = k_2 t \qquad [5]$$

Let A_1 be the amount of a particular volatile from a given sample size trapped in the first 30 min of sparge, then

$$ln[c_0/(c_0 - A_1)] = k_2 \cdot 30 \qquad [6]$$

$$c_0/(c_0 - A_1) = EXP(k_2 \cdot 30) \qquad [7]$$

$$c_0 = A_1/[1 - EXP(-k_2 \cdot 30)] \qquad [8]$$

Obtaining A_1 from GC analysis, we can calculate c_0 if we can evaluate k_2. By rearranging Equation 8 in terms of c_1, the concentration after 30 min of sparging, so that $c_0 = c_1 + A_1$,

$$A_1 = c_1 [EXP(k_2 \cdot 30) - 1] \qquad [9]$$

From Equation 9, A's are proportional to c's, and from Equation 5, the plot of $ln(c)$ vs. time will be linear with a slope of $-k_2$, so a plot of $ln(A)$ vs. time will also be linear. Peak areas from a GC chromatogram can be converted to a concentration of A_1 by considering the response factor of the GC detector; k_2 can be evaluated, and c_0 can be calculated.

According to the Clausius-Clapeyron equation, p_0 changes with temperature

$$ln(p_0) = -L/(RT) + d \qquad [10]$$

where L is the molar heat of vaporization, R is the gas constant, T is the absolute temperature, and d is an integration constant. For a homologous series,

$$L = a + bn \qquad [11]$$

where a and b are constants and n is the number of carbons in the homolog. Combining Equations 10 and 11,

$$[ln(p_0) - d]RT = -a - bn \qquad [12]$$

At isothermal conditions, T is constant and $ln(p_0)$ is a function of only n, the number of carbons.

On a given GC column under isothermal conditions, the retention times, r, are proportional to p_0's, so that $ln(r)$ is a linear function of n. Under conditions of linear temperature programming, volatile compounds move at various speeds as the temperature increases but will emerge from the column at essentially the same value of p_0, so it follows from Equation 12 that T_e, the temperature of emergence of each compound, is a linear function of n. Compounds that do not belong to a homologous series generally will not fall on whole number values of n on such plots, but can be given a value of n that reflects their retention time and ECN. For example, if a homologous series of 2-ketones is used to construct an ECN plot, aldehydes, 2-enals, 2,4-dienals, etc. can be assigned an ECN value on the 2-ketone plot according to their retention times. If the GC column chosen is nonpolar, such as methylsilicone, the interactions determining the volatility, p_0, and k_2, which is $k_1 p_0$, and the retention times and ECN are similar, and $ln(k_2)$ should be a linear function of ECN.

RESULTS AND DISCUSSION

Verification of method and theory. Figure 2 gives a plot of log(A) vs. time for six standard compounds that were added
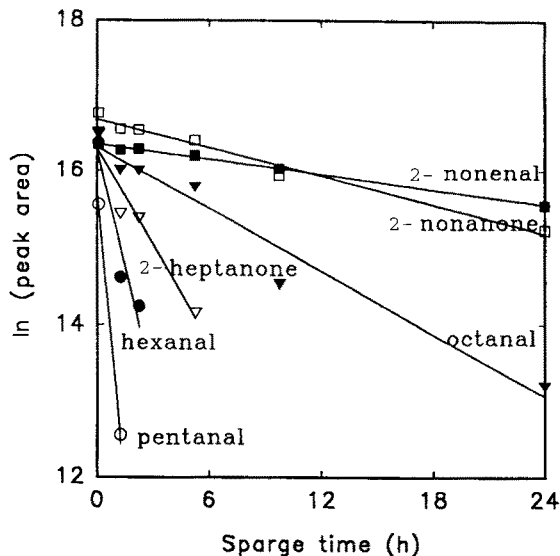
FIG. 2. Plots of sparge time vs. *In* (peak area) for six standard carbonyl compounds.

TABLE 1

Values for Volatilization Constant (k_2) and Equivalent Chain Numbers (ECN) of Various Standard Compounds

	ECN	k_2
2-Pentanone	5.00	3.309
Pentanal	5.06	2.562
t-2-Pentenal	5.55	1.579
2-Hexanone	6.00	1.284
Hexanal	6.08	1.004
t-2-Hexenal	6.56	0.577
2-Heptanone	7.00	0.420
Heptanal	7.10	0.364
t-2-Heptenal	7.60	0.250
2-Octanone	8.00	0.169
Octanal	8.10	0.135
t-2-Octenal	8.62	0.088
2-Nonanone	9.00	0.063
Nonanal	9.09	0.054
t-2-Nonenal	9.62	0.034

to volatile-stripped soybean oil and sparged continuously. As predicted by Equations 5 and 9, the plots were linear, and k_2's were obtained from the slopes. The ECNs and k_2's of 15 compounds that were determined empirically are given in Table 1. Figure 3 shows, as expected, that $\log(k_2)$ is a linear function of ECN on the methylsilicone column. The best fit of these data was

$$k_2 = 10^{-0.4206 \times (ECN) + 2.5683} \qquad [13]$$

This relation did not hold for volatiles with ECN <5.00, probably because these compounds were volatile enough to move significantly through the GC column during the 3-min hold at 30°C. Such compounds also are partly lost from the trap during the 30 min of collection and were ignored in the experiments reported here, although such compounds could be measured quantitatively by using a shorter collection time. Attempts to cool the absorbent trap with dry ice caused it to become blocked by water sparged from the oil sample during the 30-min sampling period. Compounds with ECN values >5 were not lost from the trap. This was demonstrated by connecting two traps in series and analyzing for volatiles in the second trap. Volatiles with ECN >10.00 showed little change in amount volatilized per 30 min even when sparged over a 24-h period, but their k_2 values, as well as those of unknown compounds, could be calculated from Equation 13.

The GC response factor was 1.17×10^8 peak integration units/μg for hexanal, heptanal, and 2-hexanone and was assumed to be the same for other compounds in the study. The actual concentrations of volatiles in an oil sample, c_0, were calculated from A_1's obtained in 30 min of sparge, the above constant, the k_2's, calculated from the ECN and Equation 8.

Measurement of volatiles in commercial soybean oil. Table 2 shows the identity, ECNs, k_2's, and amounts of the major

volatiles found in commercial soybean oil when fresh and after oxidation to various PVs. Most of these compounds have been reported previously in oxidized soybean oil, but the presence of significant amounts of toluene was unexpected. Smaller amounts of dimethyl- or ethylbenzene also were noted by mass spectrometry. The amounts of these aromatics were reproducible, and they were never found in unoxidized oil or adsorbent blanks. Similar aromatics have been reported in almond and plum kernel oils (32). The unknown with ECN of 7.92 had a mass spectrum suggestive of a diketone (11) and was similar to that reported in oxidized milk fat by Stark and Forss (33). Commercial soybean oil produced
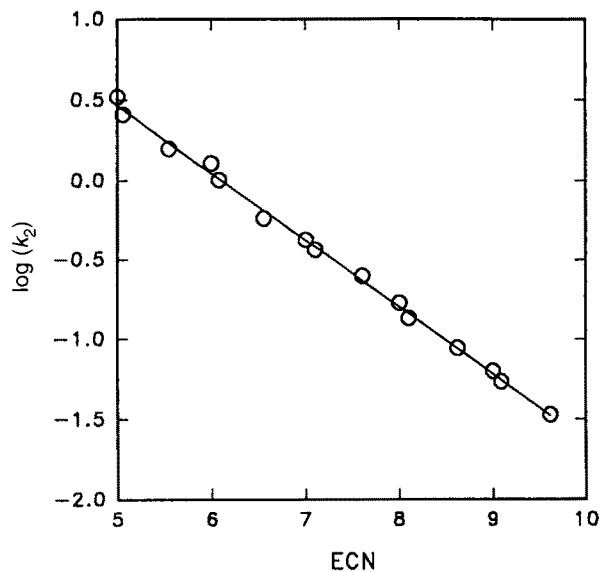

FIG. 3. Plot of log (k_2) vs. equivalent chain number (ECN) for various carbonyls.

TABLE 2
Equivalent Chain Number (ECN), k_2, and Amounts [parts per billion (ppb)] of the Predominant Volatile Compounds in Fresh and Oxidized Commercial Soybean Oils Stored at 35°C Under Fluorescent Light

Volatiles	ECN	$k_2{}^a$ (h^{-1})	Day 0 PV^b (meq/kg) 0.4	Day 4 14.3	Day 7 42.1	Day 11 73.7
			(ppb)			
1-Penten-3-one	5.00	2.919	0.25	0.64	1.92	1.64
Pentanal*,c	5.06	2.562	11.59	11.62	16.18	16.47
				(13.33)	(6.54)	(9.59)
t-2-Pentenal*	5.55	1.579	2.45	1.30	2.04	3.59
				(1.33)	(1.52)	(2.48)
Toluene	5.80	1.349	7.23	3.05	2.44	13.63
Hexanal*	6.08	1.004	18.24	45.80	70.92	74.65
				(29.33)	(44.84)	(61.85)
Heptanal*	7.10	0.364	4.05	6.51	8.35	7.12
				(6.88)	(9.09)	(9.70)
t-2-Heptenal*	7.60	0.250	14.10	72.05	170.75	270.42
				(62.58)	(226.00)	(309.05)
1-Octen-3-one	7.88	0.180	4.22	5.71	9.43	9.68
Unknown	7.92	0.173	8.75	548.28	298.60	78.00
1-Octen-3-ol	7.97	0.164	1.46	9.99	42.91	84.48
t,c-2,4-Heptadienal	8.00	0.169	6.12	22.82	57.05	76.25
2-Pentylfuran	8.09	0.146	1.84	3.33	4.85	5.45
t,t-2,4-Heptadienal*	8.12	0.143	9.90	33.43	65.31	76.76
				(24.45)	(82.02)	(118.85)
t-2-Octenal*	8.62	0.088	7.50	16.99	28.99	35.49
				(8.00)	(27.49)	(31.99)
Nonanal	9.09	0.054	24.75	81.43	96.60	66.26
t,c-2,4-Decadienal	10.94	0.009	—	—	32.37	110.98
t,t-2,4-Decadienal*	11.13	0.008	—	—	99.72	365.65
					(88.64)	(155.12)

^{a}Experimental k_2 values from Table 1 were used where available. Others were calculated by Equation 13.

^{b}Peroxide value.

^{c}To test the recovery of volatile compounds found in the oils, eight standard compounds (identified by *) were added to fresh soybean oil, in quantities simulating four-, seven-, and eleven-day stored oils. The amounts of the various compounds recovered from the simulated mixtures are presented in parentheses.

more of this compound than did the experimental varieties extracted and refined locally. Cis-3-hexenal has been reported in oxidized soybean oil (11,34,35), but no cis-3-hexenal was detected in our study, although it was demonstrated that the column used would resolve cis-3-hexenal from hexanal.

The average coefficient of variation in duplicate determinations of the volatiles was 18.6%. The coefficient tended to decrease with an increase in ECN from a value of ~30 to ~11%. The average coefficient also was greatest for the less oxidized four-day sample where the peak sizes were small.

To check the overall reliability of the method, eight compounds identified in oxidized commercial soybean oil were

added to fresh commercial soybean oil in the amounts calculated to be present in the oxidized oils at four, seven, and eleven days. Recovery of these compounds in subsequent volatile analyses of the three simulated mixtures are reported in Table 2. The amounts recovered agreed with the amounts in the oxidized oil they were intended to imitate, with an average deviation of ±26%. This error results from the summation of errors in the original and simulated determinations. The results support the assumptions made in the calculation and show that there was little artifact formation from the decomposition of hydroperoxides during the sampling at 50°C for 30 min. The deviations tended to be greatest for pentanal, the most volatile component that was measured. Probably this was the situation because slight variations in procedure allow some pentanal to escape the trap. The greatest individual deviation (57%) was recorded for 2,4-decadienal in the eleven-day sample. Probably this is because any error in measuring a volatile is exaggerated to a greater extent by the calculation as the retention time increases.

Comparison of volatiles to intensity of 2-heptanone. The flavor intensities of the individual carbonyls quantitated in these experiments can be expressed in terms of the concentration of 2-heptanone in a mineral oil emulsion that will give an equally intense flavor by using the data of Dixon and Hammond (25). The combined values for these carbonyls, which represent the predicted overall flavor intensity of the sample, can be compared with the intensity of 2-heptanone found comparable to the flavor intensity of the oxidized oil in a sensory test. The intensities of the individual carbonyls can be combined in various ways and corrected, if desired, for threshold values, but the simplest comparison is to add the individual intensities. Dixon and Hammond (25) did not provide data for some of the compounds found in our oxidized samples, so these were obtained by using their methods and are given in Table 3.

The concentrations of 2-heptanone, calculated to have the same flavor intensities as the individual compounds in Table 2, along with their sums, are given in Table 4. The sum of the calculated concentrations of 2-heptanone for each carbonyl was regarded as the predicted overall flavor intensity of the sample. The concentrations of 2-heptanone, equivalent to the flavor intensities of the individual components, are all below the threshold for 2-heptanone, which is approximately 250 ×

TABLE 3
Slopes, Intercepts, and Correlation Coefficients for Linear Fits of Log–Log Plots of the Concentrations of 2-Heptanone Perceived to Have the Same Flavor Intensities as Various Concentrations of Carbonyl Compounds

Compound	Slope	Intercept	Correlation coefficient
Diacetyl	0.463	−3.545	0.988
1-Penten-3-one	0.829	−0.047	0.994
2-Pentenal	0.650	−2.671	0.993
1-Octen-3-ol	0.541	−2.999	0.977
2-Pentylfuran	0.410	−4.046	0.978

TABLE 4
Concentrations (ppb in emulsion) of 2-Heptanone Perceived to Have the Same Flavor Intensity as the Components Isolated from Commercial Soybean Oil Oxidized at 35°C Under Fluorescent Light for Various Times

	Day			
	0	4	7	11
1-Penten-3-one	0.21	0.46	1.14	1.00
Pentanal	0.27	0.27	0.34	0.35
t-2-pentenal	0.27	0.18	0.24	0.35
Toluene	0.80	0.44	0.37	1.24
Hexanal	2.57	4.67	6.21	6.42
Heptanal	12.35	15.50	17.47	16.19
t-2-Heptenal	5.37	16.02	28.55	38.86
1-Octen-3-one	1.58	1.86	2.43	2.47
Unknown	0.05	0.05	0.05	0.05
1-Octen-3-ol	1.41	3.98	8.74	12.60
t,c-2,4-Heptadienal	17.09	29.30	42.66	48.05
2-Pentylfuran	3.54	4.51	5.26	5.52
t,t-2,4-Heptadienal	20.80	34.27	45.10	48.18
2-Octenal	5.14	8.07	10.82	12.09
Nonanal	76.58	108.18	113.70	101.90
t,c-2,4-Decadienal	—	—	15.62	26.24
t,t-2,4-Decadienal	—	—	25.09	43.30
Sum	148.0	227.8	323.8	364.8
Log (sum)	−6.83	−6.64	−6.49	−6.44

TABLE 5
Logarithms of the Volatile Data, Logarithms of the Sensory Data, and Peroxide Values (PV) of Various Types of Soybean Oil Stored at 35°C Under Fluorescent Light for Different Times

Oil source	Storage time (days)	Volatiles		Sensory		PV (meq/kg)	
		1[a]	2	1	2	1	2
A16	0	−7.02	−7.02	−6.35	−6.34	0.3	0.3
	7	−6.65	—	−6.39	—	32.2	—
	14	−6.57	−6.56	−6.26	−6.25	63.2	56.3
	21	−6.48	−6.46	−6.25	−6.23	121.7	101.0
A6	0	−6.80	−6.80	−6.25	−6.22	1.0	1.0
	7	−6.57	−6.61	−6.30	−6.32	21.4	23.4
	14	−6.45	−6.45	−6.40	−6.29	52.0	49.2
	21	−6.37	−6.41	−6.19	−6.28	88.9	96.2
A87	0	−6.78	−6.78	−6.32	−6.19	0.2	0.2
	3	−6.64	−6.64	−6.36	−6.39	11.4	11.4
	6	−6.54	−6.60	−6.29	−6.31	24.8	27.0
	9	−6.51	−6.51	−6.28	−6.36	40.1	48.3
Hardin	0	−6.98	−6.98	−6.19	−6.35	0.4	0.4
	5	−6.65	−6.63	−6.37	−6.37	23.0	22.4
	10	−6.49	−6.53	−6.27	−6.26	64.8	45.7
	14	−6.46	−6.45	−6.23	−6.24	71.2	55.2
Commercial	0	−6.88	—	−6.02	—	0.4	—
	4	−6.54	—	−6.38	—	15.1	—
	9	−6.49	—	−6.25	—	34.9	—
	14	−6.41	—	−6.21	—	81.0	—

[a]Replicate number. All data are the average of duplicate analyses. Statistical comparisons were made among oils at day 14 for volatiles, sensory, and PV analyses. No significant differences ($P < 0.05$) were noted among sensory and PV data; however, A16 had significantly fewer volatiles than did the other samples.

10^{-9} parts by volume or a log value of −6.60. Thus, if one assumes that only flavors present at concentrations above their thresholds have a flavor impact, all these compounds would have no effect on the flavor of the oxidized soybean oil. This could mean that the effect of the flavor compounds is additive. Nonanal contributed the greatest individual effect on the flavor intensity of fresh and oxidized oils, followed by *trans,trans-* and *trans,cis-*2,4-heptadienal and 2-heptenal. Although hexanal was a prominent component on the basis of peak size, its effect on flavor intensity was relatively small in these samples.

Storage tests of commercial soybean oils and soybean oils with altered fatty acid compositions. Duplicate samples of A16, A6, A87, Hardin, and commercial soybean oils were oxidized at 35°C under fluorescent light for various times. The log of the concentration of 2-heptanone necessary to duplicate the flavor of the oil as judged by the panel was compared with the log of the sum of 2-heptanone concentrations calculated to be equivalent to the individual flavor compounds in the sample. Log values were used to compare flavor intensities because sensory intensities are linearly related to the log of the concentration of flavor compounds (22). Each of the duplicate oil samples, except the commercial sample, was analyzed in duplicate sensory and volatiles tests, and the average values are presented in Table 5. The fatty acid composition of these oils is shown in Table 6. As oxidation proceeded, the logarithmic values of both the volatiles and the sensory tests became less negative, as expected. Duplicate determinations agreed quite closely; the average deviation between duplicates of the volatiles analysis was 0.018 log units, and that of the sensory panel 0.049 log units. The sensory values were larger than the volatiles values in every instance. Although

the volatiles analysis had a fairly large error, it was 2.7-fold smaller than the sensory error, so the volatiles analysis was accurate enough for the comparisons made in this study. The average difference between the sensory and volatiles values was 0.38 units, which corresponds to a 2.4-fold difference in 2-heptanone concentration. This difference between sensory and volatile values tended to be the greatest at zero time and diminish as oxidation proceeded. If we bear in mind that the threshold value for 2-heptanone is approximately −6.60, it is obvious that the zero- and one-week values for the headspace analyses were below threshold. This was confirmed by tasting mixtures of appropriate concentrations of these components in mineral oil emulsions. The sensory values for the oils at zero time were well above threshold, and in most instances, became less intense at about one week before increasing again as oxidation proceeded. The volatiles values in Table 5 were correlated with the sensory values after removal of the data for the fresh oils and for A6, which had the greatest variations in the sensory data. The correlation coefficient of the plot was 0.72 with a slope of 1.027 ± 0.151. A slope of 1 would be expected if the volatiles accounted for the change in flavor intensity.

The average difference of 0.38 log units between the sensory and volatiles values suggests that the major volatile oxidation products that were measured accounted for less than half of the flavor of the soybean oil samples. Probably, this discrepancy can be attributed to the presence of some un-

TABLE 6
Fatty Acid Compositions (%) of the Soybean Oils

Oil source	16:0	18:0	18:1	18:2	18:3
Commercial	11.3	4.2	25.1	53.1	6.3
Hardin[a]	10.7	3.7	25.7	54.5	5.7
A6[a]	8.4	27.5	21.5	39.5	4.0
A16[a]	10.8	5.8	32.5	48.7	2.0
A87[a]	10.3	4.3	29.6	54.2	2.0

[a]From Liu and White (Ref. 27).

known and unmeasured flavor in fresh soybean oil because known compounds do not account for the flavor. Presumably, in the headspace test used here, these unknown compounds were either not volatile enough or present in too small amounts to be detected. Alternatively, the difference in the headspace and sensory results might be attributed to a synergistic interaction among the volatiles, so that the mixture had a much stronger flavor than would be expected from simple summation of the flavors of the individual components. Day et al. (20) presented evidence that such positive interactions sometimes occur.

The 2-heptanone standards used in the sensory tests ranged from near threshold (log value −6.60) to very strong (log value −5.40). The flavor intensity of the oil samples, regardless of their stage of oxidation, clustered around the second weakest standard (log value −6.30), and none exceeded the third standard (log value −6.00) in intensity.

Table 7 shows the change in mole percentage of the various volatile components in A6 when exposed to air and fluorescent light. In the beginning, pentanal and hexanal were present in the greatest concentrations, but as oxidation proceeded, the percentage of 2-heptenal increased. Light oxidation favors 2-heptenal formation (5). 1-Octen-3-ol increased in concentration during oxidation; toluene dropped precipitously. The unknown with ECN 7.92 could not be calculated

TABLE 7
Mole Percentage of Individual Volatiles in the Total Known Volatiles from A6 Soybean Oil Oxidized Under 1937 Lux of Fluorescent Light at 35°C for Various Times

Compound	Weeks			
	0	1	2	3
1-Penten-3-one	0.11	0.25	0.44	0.52
Pentanal	10.22	8.31	4.58	5.12
t-2-Pentenal	0.42	0.55	0.77	0.92
Toluene	9.61	0.46	0.20	0.14
Hexanal	41.87	21.40	17.23	14.59
Heptanal	3.70	1.83	1.26	0.73
t-2-Heptenal	9.45	29.19	33.27	31.64
1-Octen-3-one	1.06	1.85	1.63	1.23
1-Octen-3-ol	0.63	4.74	7.96	16.89
t,c-2,4-Heptadienal	1.85	5.25	6.77	4.55
2-Pentylfuran	4.96	4.97	3.89	2.09
t,t-2,4-Heptadienal	3.85	6.13	7.51	7.35
t-2-Octenal	3.12	4.81	3.97	2.97
Nonanal	9.19	9.25	8.09	4.50
t,c-2,4-Decadienal	0.00	0.92	1.94	4.68
t,t-2,4-Decadienal	0.00	0.00	0.48	2.07

TABLE 8
Mole Percentage of Known Volatile Compounds in Soybean Oils with Various Fatty Acid Composition Oxidized (for fourteen days) Under 1937 Lux of Fluorescent Light at 35°C

Compound	Oil source				
	Commercial	Hardin	A6	A16	A87[a]
1-Penten-3-one	0.13	0.43	0.45	0.15	0.39
Pentanal	1.85	2.18	4.73	3.24	3.06
t-2-Pentenal	0.29	0.78	0.80	0.39	0.39
Toluene	1.02	3.65	0.20	0.91	0.45
Hexanal	7.97	10.65	17.78	17.82	1.28
Heptanal	0.83	0.80	1.39	1.06	0.94
t-2-Heptenal	24.62	29.08	33.27	34.70	35.58
1-Octen-3-one	1.10	0.90	1.63	1.33	1.19
1-Octen-3-ol	5.73	12.98	7.96	10.93	15.76
t,c-2,4-Heptadienal	8.03	5.58	6.68	4.14	3.32
2-Pentylfuran	0.77	0.79	4.02	1.79	1.33
t,t,2-4,-Heptadienal	8.35	9.89	7.75	5.25	5.89
t-2-Octenal	5.28	3.21	4.09	4.05	3.31
Nonanal	10.04	6.46	8.35	8.39	8.02
t,c-2,4-Decadienal	18.95	9.36	1.94	4.45	15.03
t,t-2,4-Decadienal	5.03	2.73	0.48	1.40	4.07

[a]Oxidized for nine days.

because its molecular weight is unknown, so it is not given in Table 7, but its proportion declined with time. In general, the changes with time noted for A6 were similar for other samples.

Table 8 compares the mole percentages of the various volatile components in the oils at fourteen days (nine days for A-87) of oxidation under fluorescent light. Assuming that nonanal comes from oleate, that 1-penten-3-one, 2-pentenal, toluene, and 2,4-heptadienal come from linolenate and that other volatiles come from linoleate (36), then the total volatiles from linolenate in the low-linolenate oils (A6, A16, and A87) were lower than these volatiles in Hardin and the commercial oil, which contain typical linolenate concentrations for soybean oil. But the amounts in the low-linolenate oils were not as low as would be expected from the relative rates of oxidation reported for oleate, linoleate, and linolenate. The amount of nonanal was consistently greater than expected from the relative oxidation rate of oleate. The partition of linoleate hydroperoxides into hexanal, 1-octen-3-ol, pentylfuran, and 2,4-decadienal varied widely from oil to oil, but the causes of these variations are not clear.

It is difficult to compare the relative stability of the oil samples because the amounts of these special oils were limited, and they were oxidized for different periods in an attempt to optimize the changes in volatiles. At the fourteen-day oxidation period common to A16, A6, Hardin, and the commercial samples (Table 5), A16 had significantly fewer volatiles than the other samples, but there were no significant differences in PV or sensory values. The volatiles and PV measurements were related, however, as demonstrated by a correlation of 0.85 between the log of the 2-heptanone concentrations and the log PV listed in Table 5.

Liu and White (27) found A16 to be more stable than A6 and Hardin after 15-days' storage at 60°C in the dark, when

flavor quality and intensity were measured by sensory panels. PV measurements were significantly less (*P*<0.05) for both A6 and A16 oils than for Hardin. Similarly, in testing Hardin, A87, and A16, Mounts *et al.* (37) found A16 to be superior in PV and volatiles tests after 60°C storage in the dark for eight days, but sensory results were varied. The soybean oils in their tests were hexane-extracted and had added citric acid, rather than cold-pressed with no citric acid addition in the current study and in the study of Liu and White (27). Despite the processing differences, A16 tended to be more stable than the other oils in all three studies.

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