Analysis of Vegetable Oil Volatiles by Multiple Headspace Extraction[1]

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Quantitative determination of the volatiles produced from oxidized vegetable oils is an important indicator of oil quality. Five vegetable oils, low-erucic acid rapeseed, corn, soybean, sunflower and high oleic sunflower, were stored at 60°C for four and eight days to yield oils with several levels of oxidation. Peroxide values of the fresh oils ranged from 0.6 to 1.8 while those of the oxidized oils were from 1.6 to 42. Volatile analysis by the multiple headspace extraction (MHE) technique, which includes a pressure and time controlled injection onto the gas chromatography (GC) column (a chemically bonded capillary column), was compared with that obtained by static headspace gas chromatography (SHS-GC). Several volatile compounds indicative of the oxidation of polyunsaturated fatty acids from the vegetable oils were identified and measured by MHE; pure compounds of twelve major volatiles also were measured by MHE, and peak area was determined. Multiple extractions of the oil headspace provided a more reproducible measure of volatile compounds than was obtained by SHS-GC. Concentration of all volatiles increased with increased oxidation as measured by peroxide value of the oil.

KEY WORDS: Canola oil, corn oil, headspace, high oleic sunflower oil, oxidation, peroxide value, quantitation, soybean oil, static, sunflower oil.

Volatile compounds formed during vegetable oil storage have been used as a measure of the oxidation; quantitation has varied with the method used for volatile analysis (1-3). However, accurate measurement of vegetable oil volatiles by static headspace has not been reported. Static headspace analysis depends on an equilibrium of the partition between the volatiles in the sample and the surrounding gas inside a closed vial. Therefore, by including the partition coefficient into a calibration factor, an accurate concentration could be determined. Kolb (4) and Kolb *et al.* (5) previously developed a stepwise gas extraction at equal time intervals called multiple headspace extraction (MHE) to quantitate the volatile compounds from liquid and solid materials; this method used the same equipment as for single static headspace analysis (SHS) and was not dependent upon the sample matrix (6-7). Suzuki *et al.* (8) measured solvents in adhesive tape while Uhler and Miller (9) measured volatile halocarbons in butter with multiple headspace extraction. The use of this extraction technique of multiple injections from a single headspace vial to accurately determine the volatile constituents formed in vegetable oils is reported here.

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MATERIALS AND METHODS

Materials. Twelve pure compounds representing the major volatiles previously identified in vegetable oils (10,11) were obtained from Bedoukian Research Inc. (Danbury, CT): propanal, pentane, pentanal, pentanol, hexanal, 2-pentenol, 2/3-hexenal, 2-heptenal, octen-3-ol, 2,4-heptadienal, nonanal and 2,4-decadienal. Each compound was diluted in hexadecane and 50 to 150 ng of each standard was measured into the sample vials for volatile analysis.

Refined, bleached and deodorized corn, soybean, low erucic acid rapeseed (LEAR), sunflower and high oleic sunflower oils were used in this study. Samples of these oils were stored in glass bottles with air in the headspace at 60°C using a modified Schaal oven method (12); samples were removed at 4 days and 8 days. Peroxide values were determined by AOCS method Cd 8-53 (13).

Methods. Volatile analysis was accomplished by multiple headspace extraction using a Perkin Elmer 2000 GC (Norwalk, CT) equipped with a Perkin Elmer HS100 headspace sampler. Oil samples (0.5g) were weighed into 25 mL vials, sealed and heated at 90°C for 30 min for the thermostat time. After the vial was pressurized for 30 sec, the sample was injected onto the gas chromatography (GC) column for a 30 sec period. The vial was vented and equilibrium established before the next injection. Each vial was sampled 3 times for multiple extraction, and each sample was replicated 3 times for a statistical analysis. The GC analyses were made using a DB-1701 capillary column (30m × 0.32mm) (J&W, Cardova, CA) by temperature programming from -20°C to 250°C at 5°C/min. The carrier gas was helium with a velocity of 28 cm/sec. The mode of operation was splitless.

The headspace temperature was very critical for analyzing vegetable oils. At temperatures above 90°C, decomposition of hydroperoxides resulted in generation of volatile compounds with each subsequent thermostat time and peak size increased instead of decreasing after each extraction. If the temperature was too low, sample size would be so small that GC peaks representing the individual volatiles would not show up on the second or third extraction. The temperature that gave the best results was 90°C.

Single headspace sampling (SHS) was accomplished using the Perkin Elmer HS100 headspace sampler with one extraction from each vial. Conditions were the same as multiple headspace, and each sample was replicated 3 times. The volatiles were quantitated by standard additions of each volatile compound analyzed (14).

Compositional data of the oils were made by GC analysis of the fatty acid methyl esters by AOCS method Ce 1-62 (13) using a SP-2330 column (0.32mm × 30m) (Supelco, Bellefonte, PA).

RESULTS AND DISCUSSION

Multiple headspace extractions involving the repeated withdrawal of volatile compounds in the headspace of a solution can be related to a liquid-liquid extraction as with a series of separatory funnels which depends on the partition coefficients of the two liquids. The liquid in a sample vial is at equilibrium with the gas phase above it, and the ratio of the concentration of the volatile component in the gas phase to the concentration of the volatile in the liquid corresponds to the partition coefficient of the component between the two phases. A partial sampling of the total gas volume is removed from the vial with each extraction and is eluted onto the GC column; the corresponding peak represents that concentration. The ratio between the two phases or the partition coefficient remains the same after equilibration while the concentration of the compound in both phases is smaller than it was originally. When the vial is sampled again, the corresponding peak will be smaller than the first. After repeated extractions, the GC peak gets smaller as the concentration of volatile decreases. The total amount of the volatile equals the sum of all the peaks. The repeated sampling of the equilibrium headspace follows the mathematics of a first-order reaction (5). The decrease in concentration (C) with time (t) is proportional to the overall concentration: $-dC/dt = kC$. The concentration at any time (C_i) depends on the initial concentration (C_0) and the exponent k which includes the partition coefficient: $C_i = C_o e^{-kt}$.

With uniform sampling and equal time intervals of headspace extractions, time can be replaced by the

TABLE 1

Fatty Acid Composition of Vegetable Oils

Fatty acid	Corn	LEAR[a]	Soybean	Sunflower	High oleic sunflower
		(percent)			
C 16:0	11.4	4.8	10.8	6.8	9.9
C 18:0	1.9	1.7	3.8	3.9	8.3
C 18:1	24.9	61.0	22.9	14.2	82.5
C 18:2	56.8	22.4	55.5	74.5	9.3
C 18:3		9.0	8.1		
C 22:1		1.2			

[a]Low erucic rapeseed.

TABLE 2

MHE Volatile Analysis of Vegetable Oils

		Volatile compound (ppb)[a]											
		1	2	3	4	5	6	7	8	9	10[b]	11	12[b]
Corn sample													
Day	(PV)*												
0	(0.8)		0.8	3.9		7.8			0.8			0.2	
4	(1.8)	2.0	5.0	10.9		29.9		1.2	6.2	0.4		1.3	14.0
8	(3.0)	8.9	53.4	24.0		85.5		5.7	18.4	0.7		3.4	16.0
Low erucic rapeseed sample													
Day	(PV)												
0	(1.8)	4.2	2.4	5.5	3.8	11.4		1.4	2.2		7.0	1.8	
4	(3.5)	10.9	39.9	59.5	4.6	64.0	5.8	5.7	21.2	3.1	60.3	8.6	14.8
8	(17.3)	11.9	132.2	48.4	9.4	86.0	11.8	11.1	27.6	1.7	106.8	16.5	26.9
Soybean sample													
Day	(PV)												
0	(0.6)		1.0	1.9		2.8			0.4		0.3		0.3
4	(1.6)	3.6	33.2	19.1		33.8	3.3	4.8	16.7	0.8	19.2	1.8	7.5
8	(4.5)	4.5	91.9	44.0		70.6	3.6	6.4	30.1	1.1	50.0	3.4	16.4
Sunflower sample													
Day	(PV)												
0	(0.8)		5.5	2.9		7.5			2.8				1.3
4	(19.6)	2.8	281.4	40.1	1.6	118.9		7.3	34.8	1.2		1.0	30.4
8	(41.6)	4.5	605.6	61.3	2.7	183.9		13.7	52.2	2.2		2.4	89.7
High oleic sunflower sample													
Day	(PV)												
0	(0.6)	1.4	2.8	4.1		5.8			1.5			1.1	0.5
4	(3.2)	2.0	21.4	7.6	1.2	18.7		5.8	7.9			10.9	9.4
8	(6.7)	4.0	134.4	20.5	3.4	35.0		10.4	20.7			23.8	24.5
CO[c]		6.7	6.1	5.1	5.6	3.9	6.6	5.7	6.2	7.0	3.0	8.4	8.7

*Peroxide value (PV) in parentheses.

[a]1)propanal 2)pentane 3)pentanal 4)pentanol 5)hexanal 6)2-pentenol 7)2/3-hexenal 8)2-heptenal 9)octen-3-ol 10)2,4-heptadienal 11)nonanal 12)2,4-decadienal.

[b]Includes both *trans,trans* and *trans,cis* isomers.

[c]Coefficient of variation (average) (CO).

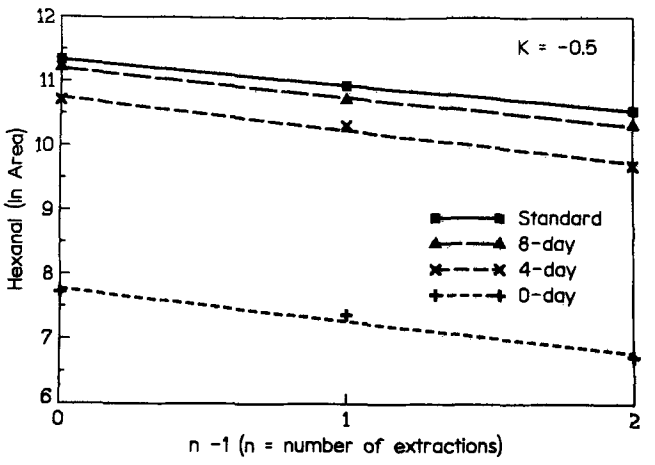

FIG. 1. Partition coefficient of hexanal by MHE (standard and in soybean oil).

number of extractions (n). GC peak area is proportional to concentration, and C_0 can be replaced by the first area (A_1). Time can be replaced by n-1 and the total area (A_i) is: $A_i = A_1 e^{-k^*(n-1)}$.

From the logarithim of the equation: $\ln A_i = -k^* (n-1) + \ln A_1$, the linear regression can be accomplished where $y = \ln A_1$ and $x = k^*$, and k^* can be determined from the GC data from only 3 or 4 extractions. The total amount of volatile compound present in the sample is obtained from the sum of all the peak areas and can be written as the geometric progression: Sum $A_i = A_1 [1 + e^{-k^*} + e^{-2k^*} + e^{-(n-1)k^*}]$ which can be simplified as, Sum $A_i = A_1/1\text{-}e^{-k^*}$.

Hexanal, an important oxidation product from polyunsaturated fatty acids, was used as the compound to demonstrate how the concentrations were calculated (5). After 3 headspace extractions of hexanal in hexadecane, the total area was calculated for the standard by a linear regression of the natural log of the peak areas and the partition coefficient determined from the slope of the linear equation. The partition coefficient of hexanal in the initial oil and the stored oils is shown by the slope 0.5 in Figure 1. The total hexanal peak area from the initial soybean oil and the storage-damaged oils was calculated by the same method as used for the standard: hexanal (standard) with number of extractions (n) 1, 2, and 3 with areas of 84,208, 56,080, and 38,012 respectively, totaling 256,012; and hexanal in oil with number of extractions (n) 1, 2, and 3 with areas of 74,254, 45,016, and 18,377 respectively, totaling 147,766.

The weight of the hexanal standard was 61ng; the weight of the hexanal in the oil was calculated to be 35ng. The concentration of each of the twelve volatiles in the five oils was determined for 0, 4 and 8 day storage times.

TABLE 3

Volatile Analysis of Vegetable Oils by SHS

		Volatile compound (ppb)[a]											
		1	2	3	4	5	6	7	8	9	10[b]	11	12[b]
Corn													
Day	(PV)*												
0	(0.8)		0.8	1.9		5.2			0.8			0.3	
4	(1.8)	0.9	4.0	9.1		23.9		0.1	7.4	0.0		1.7	8.8
8	(3.0)	6.2	69.6	20.1		80.2		0.6	20.6	0.1		2.3	5.4
Low erucic rapeseed													
Day	(PV)												
0	(1.8)	4.0	1.5	3.4	0.2	8.5		0.1	1.7		3.9	1.8	
4	(3.5)	6.5	47.8	31.3	0.3	59.3	3.8	0.5	18.2	0.2	43.3	9.5	7.4
8	(17.3)	11.7	119.9	45.3	8.5	70.9	7.4	1.7	25.5	0.1	68.6	13.6	14.4
Soybean													
Day	(PV)												
0	(0.6)		0.5	0.8		1.6			0.3		0.6		0.6
4	(1.6)	3.0	7.8	18.6		32.7	2.4	0.5	10.3	0.1	17.4	2.2	5.6
8	(4.5)	3.9	95.2	41.0		54.2	1.9	0.7	28.5	0.1	42.8	0.6	12.3
Sunflower													
Day	(PV)												
0	(0.8)		5.7	2.8		7.2			3.4				1.0
4	(19.6)	3.4	258.3	29.9	0.1	86.1		0.6	28.8	0.1		0.9	23.9
8	(41.6)	5.7	296.7	46.0	0.1	146.2		1.3	49.5	0.1		2.6	60.3
High oleic sunflower													
Day	(PV)												
0	(0.6)	1.2	2.4	2.8		4.8			1.5			0.9	0.8
4	(3.2)	1.9	23.2	5.9	0.1	20.6		0.6	18.3			10.6	7.4
8	(6.7)	3.6	126.2	24.9	0.2	43.6		1.5	31.8			18.1	14.6
CO[c]		6.5	4.7	8.5	12.2	4.4	11.9	8.5	7.3	15.5	9.7	8.7	9.4

See Table 2 for footnotes.

The fatty acid composition of each test oil is presented in Table 1. Oils with the highest linoleate concentration had greater amounts of pentane and hexanal formed from the decomposition of 13-linoleate hydroperoxide (11,15). Both pentane and hexanal increase to the greatest extent during the storage of sunflower oil, followed by the oxidation of corn, soybean and high oleic sunflower oil (Table 2). LEAR oil and high oleic sunflower have the greatest amount of oleate; therefore nonanal formed from the decomposition of oleate hydroperoxides is higher in these two oils (16). 2,4-Heptadienal, the product from linolenate oxidation, is formed only in LEAR and soybean oil.

Changes in the concentration of the individual volatile compounds that were formed during storage of each oil were measured by the MHE method (Table 2). Concentration of all volatiles increased with oxidation except for pentanal and octen-3-ol in LEAR oil. Pentane showed the greatest increase in each oil except for high oleic sunflower oil in which hexanal showed the greatest increase.

When the volatiles were measured by the single headspace technique, the volatiles increased with storage except for decadienal in corn oil, octen-3-ol in LEAR oil and 2-pentenol and nonanal in soybean oil (Table 3). When individual compounds were compared using both methods, there was little difference in the quantities of the compounds present in the lesser amounts. Comparing the major compounds, there were differences specific to certain oils. Pentane was present in about the same concentration for all oils by each method except for sunflower oil; i.e. pentane concentration after 8 days was 2 times greater using MHE than with SHS. Pentanal and hexanal were 1.7 times higher in high oleic sunflower oil by MHE than by SHS. Heptenal was about the same concentration in all oils by either method. 2,4-Decadienal showed 1.5 greater concentration in sunflower oil by MHE than by SHS. Multiple headspace extraction showed a greater reproducibility than single headspace sampling, the average coefficient of variation with MHE was lower for all volatiles studied except for pentane and propanal.

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