Comparison of Gas Chromatographic Methods for Volatile Lipid Oxidation Compounds in Soybean Oil

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To develop new knowledge on undesirable flavors affecting the quality of foods containing polyunsaturated lipids, we investigated the volatiles in soybean oil oxidized at different conditions by three capillary gas chromatographic methods: (a) direct injection (5 min heating at 180 C); (b) static headspace (20 min heating at 180 C, pressurizing for one min), and (c) dynamic headspace (purging 15 min at 180 C onto a porous polymer trap, desorbing from trap for five min). A fused silica column was used with bonded polymethyl and phenyl siloxane phase. At peroxide values between 2 and 10, the major volatile products found in soybean oil by the three methods were pentane, hexanal, 2-heptenal, 2,4-heptadienal and 2,4-decadienal. The intensities of each volatile compound varied with the analytical methods used.

Various gas chromatographic (GC) methods have been reported to analyze volatile products from the oxidative deterioration of vegetable oils (1-9). The advantages and disadvantages of methods to analyze volatiles have been discussed (10), but no work has been published comparing different methods for vegetable oils of known storage histories. This paper reports a study of three capillary GC methods (direct injection, dynamic headspace and static headspace) to analyze the volatiles formed in soybean oil samples oxidized to different levels. The GC profiles obtained by each method were evaluated to better understand how volatile analyses can be used to assess quality of vegetable oils and to predict flavor stability.

EXPERIMENTAL

Samples of refined, bleached, deodorized (RBD) soybean oil were stored with air in the headspace at room temperature in the dark and oxidized to different peroxide values (PV) which were determined by AOCS method Cd 8-53 (11).

Volatile compounds in oxidized oil samples were analyzed by three techniques as follows:

Direct injection. An oil sample of two µl was introduced onto glass wool positioned in the glass liner of a Perkin Elmer Sigma B GC injector (Norwalk, Connecticut) at 180 C (8). Volatile compounds generated from the oil were eluted onto a Durabond-5 chemically bonded capillary column, 60 m × 0.32 mm i.d. with one micron film thickness (J&W Scientific, Rancho Cordova, California). The GC oven was held at -65 C for five min while the injector was in the splitless mode. The temperature was then programmed to 270 C at 5 C/min. The glass liners were removed after each sample; a clean liner with fresh glass wool was conditioned in the injector at 250 C for 30 min before introducing the next sample.

Dynamic headspace. Oil samples of five g were heated to 180 C and purged with He for 15 min in a Tekmar Concentrator (Cincinnati, Ohio) (9). The volatiles were collected on a trap containing porous polymer Tenax GC adsorbent (Anspec Co., Ann Arbor, Michigan), and then thermally desorbed at 220 C onto a 60-m Durabond-5 capillary column while the GC was in the splitless mode for 5 min. The GC oven temperature was held at -65 C during the five-min desorb time and then programmed to 270 C at 5 C/min. During the GC run the trap was heated to 270 C and backflushed with He to remove any compounds left on the trap. A blank with a clean sample tube was run between samples.

Static headspace. An oil sample of 0.5 g was sealed in a 10-ml clean vial and heated to 180 C for 20 min in the headspace magazine of a Perkin Elmer headspace sampler, Model HS-6 attached to a Perkin Elmer Sigma 3B GC (7). The headspace sampler was placed into the injection position and pressurized for one min. The volatiles were automatically transferred onto a Durabond-5 capillary column (30 m × 0.32 mm i.d. with one micron film thickness) after the He flow was interrupted. The GC oven was held at 0 C for 10 min and programmed to 250 C at 5 C per min. Consecutive GC

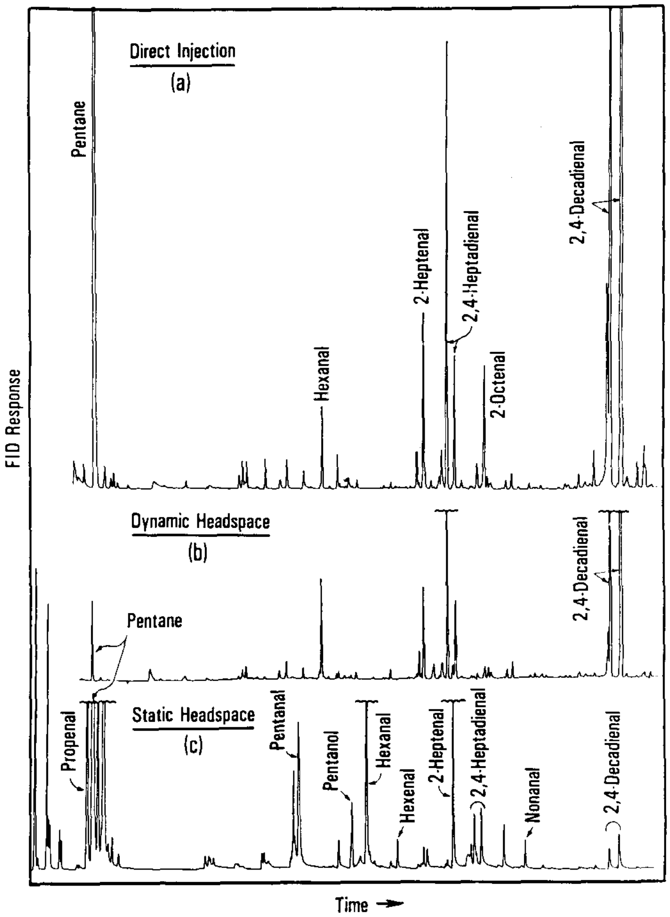

FIG. 1. Comparison of three GC methods for volatile analyses of oxidized soybean oil (PV 9.5) heated to 180 C. (a) Direct injection; (b) dynamic headspace, (c) static headspace.

TABLE 1

Headspace Analysis of Oxidized Soybean Oil[a] by Three Methods: Direct Injection (DI), Dynamic Headspace (DHS) and Static Headspace (SHS)

Volatile compound	PV=1.7						PV=4.9			
	DI		DHS		SHS		DI		DHS	
	Area[b]	Rel%	Area	Rel%	Area	Rel%	Area	Rel%	Area	Rel%
Acrolein	9.2	1.5	0.0	0.0	6.6	8.8	2.3	0.9	0.3	0.2
Propanal	4.3	0.7	0.0	0.0	7.1	9.5	3.5	0.2	0.0	0.0
Pentane	127.6	20.1	1.9	9.7	23.7	31.6	178.4	23.3	13.9	8.3
Pentene	2.3	0.4	0.1	0.4	0.2	0.3	1.7	0.2	1.2	0.7
Butanal	1.1	0.2	0.0	0.0	0.2	0.3	0.9	0.1	0.2	0.1
1-Penten-3-ol	3.5	0.6	0.4	2.0	1.4	1.9	2.0	0.3	1.0	0.6
Pentanal	5.9	0.9	0.4	2.2	6.0	8.0	3.3	0.4	1.5	0.9
2-Pentenal	1.6	0.3	0.0	0.0	0.5	0.8	2.0	0.3	1.2	0.7
1-Pentanol	4.2	0.7	0.0	0.0	1.6	2.1	3.2	0.4	0.8	0.5
Hexanal	10.4	1.6	1.6	8.4	8.0	10.7	16.3	2.1	5.9	3.5
Octane	4.6	0.7	0.2	0.7	0.3	0.4	5.2	0.7	0.7	0.4
2/3-Hexenal	1.3	0.2	0.3	1.3	0.5	0.7	3.1	0.4	1.0	0.6
Octene	1.1	0.2	0.2	0.6	0.1	0.1	0.5	0.1	0.5	0.3
Heptanal	2.4	0.4	0.3	1.4	0.2	0.3	1.5	0.2	0.8	0.4
2-Heptenal	19.3	3.0	1.8	8.4	3.1	4.1	10.3	1.4	9.9	5.9
1-Octen-3-ol	4.7	0.7	0.2	1.0	0.0	0.0	3.4	0.5	1.7	1.0
2,4-Heptadienal (*trans,trans*)	18.6	2.9	1.3	6.7	0.5	0.7	31.3	4.1	18.1	10.8
Pentyl furan	1.3	0.2	0.2	0.8	0.6	0.8	0.0	0.0	0.5	0.3
Octanal	0.7	0.1	0.1	0.4	0.2	0.3	0.0	0.0	0.0	0.0
2,4-Heptadienal (*trans,cis*)	17.8	2.8	1.1	5.8	0.9	1.2	14.0	1.8	9.4	5.6
2-Octenal	10.8	1.7	0.5	2.4	0.5	0.7	9.2	1.2	1.0	0.6
Nonanal	4.6	0.7	0.5	2.8	0.2	0.3	10.8	1.4	1.0	0.6
2-Nonenal	1.8	0.3	0.4	2.0	0.1	0.1	2.2	0.3	0.5	0.3
2-Decenal	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.2	0.0	0.0
2,4-Decadienal (*trans,trans*)	70.8	11.2	1.1	5.7	0.1	0.1	100.4	13.1	24.6	14.7
2,4-Decadienal (*trans,cis*)	148.1	23.3	2.3	12.0	0.3	0.4	212.9	27.8	49.0	29.8
Others	156.2	24.6	4.8	24.9	12.2	15.8	152.8	18.6	22.1	13.2
Coeff of var[d]	7.6%		6.8%		4.5%		6.5%		5.8%	
Total area[e]	634.8		19.8		75.1		765.0		166.8	

[a] Refined, bleached, deodorized; no additives; stored in dark, in air, room temp.

[b] Area = peak area (X10^{-3}).

[c] LnOOH, linolenate hydroperoxide; LoOOH, linoleate hydroperoxide; OlOOH, oleate hydroperoxide.

runs could be made without cleaning between samples because no memory effects were observed.

Duplicates were run for each sample by all methods, and the coefficient of variation was determined for each volatile studied. Dodecane was used as an internal standard.

RESULTS AND DISCUSSION

To develop new knowledge on preventing the formation of undesirable flavors in foods containing polyunsaturated lipids, the quantities of selected volatile lipid oxidation products were investigated in samples of known processing and storage histories. Gas chromatographic analysis of the volatiles generated from a soybean oil oxidized to a PV of 9.5 as obtained by the direct injection, dynamic and static headspace techniques are shown in Figure 1. Major peaks labeled on the chromatograms have been identified previously by GC-MS (7,9). While the same volatile compounds are shown on each chromatogram, the GC profiles are different because of the different collection methods. For example, by the direct injection method, pentane and the isomers of heptadienal and decadienal are found in the largest quantities (Fig. 1a). Thermal decomposition of volatile precursors in the injector may account for the higher concentrations of the carbonyl compounds. By the dynamic headspace method, major volatile components include relatively less pentane and large quantities of the isomers of heptadienal and decadienal (Fig. 1b). With this technique, the lower-boiling compounds may be lost during the purging cycle while other components such as hexanal, heptadienal and decadienal may be concentrated in the trap. By the static headspace

TABLE 1 (continued)

Headspace Analysis of Oxidized Soybean Oil[a] by Three Methods: Direct Injection (DI), Dynamic Headspace (DHS), Static Headspace (SHS)

Volatile compound	PV=4.9		PV=9.5						
	SHS		DI		DHS		SHS		
	Area	Rel%	Area	Rel%	Area	Rel%	Area	Rel%	Origin[c] (11)
Acrolein	21.1	10.2	15.6	0.9	1.3	0.2	16.5	6.6	16-LnOOH
Propanal	18.8	9.1	5.2	0.3	0.0	0.0	31.5	12.6	16-LnOOH
Pentane	61.6	29.8	443.2	25.5	20.3	3.1	58.3	23.3	13-LoOOH
Pentene	0.2	0.1	6.9	0.4	2.0	0.3	0.3	0.1	
Butanal	0.8	0.4	10.4	0.6	9.8	1.5	1.8	0.7	?
1-Penten-3-ol	6.6	3.2	10.2	0.6	3.3	0.5	8.5	3.4	
Pentanal	15.4	7.4	5.2	0.3	7.8	1.2	16.8	6.7	13-LoOOH
2-Pentenal	1.5	0.7	6.7	0.4	7.5	1.2	1.8	0.7	13-LnOOH
1-Pentanol	4.7	2.3	3.5	0.2	2.6	0.4	6.0	2.4	13-LoOOH
Hexanal	23.4	11.3	13.8	0.8	29.4	4.5	37.3	14.9	13-LoOOH
Octane	0.9	0.4	5.6	0.1	0.1	0.1	1.0	0.4	10-OlOOH
2/3-Hexenal	1.5	0.7	1.4	0.3	4.6	0.7	2.0	0.8	13-LnOOH
Octene	0.2	0.1	1.4	0.1	1.4	0.2	0.5	0.2	
Heptanal	0.8	0.4	10.4	0.1	3.9	0.6	1.0	0.4	?
2-Heptenal	9.6	4.6	34.6	2.0	36.6	5.6	12.5	5.0	12-LoOOH(?)
1-Octen-3-ol	0.1	0.1	10.4	0.6	5.9	0.9	0.5	0.2	10-LoOOH(?)
2,4-Heptadienal (*trans,trans*)	1.2	0.6	72.7	4.2	72.6	11.1	3.0	1.2	12-LnOOH
Pentyl furan	1.3	0.6	0.0	0.0	5.2	0.8	0.8	0.3	?
Octanal	0.7	0.3	0.0	0.0	0.0	0.0	0.5	0.2	11-OlOOH
2,4-Heptadienal (*trans,cis*)	3.1	1.5	26.0	1.5	29.6	4.5	3.8	1.5	12-LnOOH
2-Octenal	1.3	0.6	22.5	1.3	3.3	0.5	1.3	0.5	?
Nonanal	1.3	0.6	6.9	0.4	4.5	0.7	1.5	0.6	9-/10-OlOOH
2-Nonenal	0.1	0.1	5.8	0.3	1.3	0.2	0.0	0.0	?
2-Decenal	0.1	0.1	5.8	0.3	0.0	0.0	0.0	0.0	9-OlOOH
2,4-Decadienal (*trans,trans*)	0.2	0.1	266.6	15.4	117.0	17.9	0.5	0.2	9-LoOOH
2,4-Decadienal (*trans,cis*)	1.0	0.5	526.3	30.3	220.4	33.7	1.2	0.6	9-LoOOH
Others	29.3	14.0	228.8	13.1	63.7	9.6	41.2	16.0	
Coeff of var[d]	4.8%		7.5%		6.0%		5.4%		
Total area[e]	206.8		1745.9		654.1		250.1		

[d] Coefficient of variation, (from duplicate GC data) average of all volatile compounds.
[e] Total integrated GC peak area.

method, the low molecular weight or low boiling volatile compounds such as propanal and pentane are found in much larger relative quantities than the heptadienal and decadienal isomers (Fig. 1c).

Quantitative analyses of the volatile compounds by the three GC methods are compared in Table 1. The amounts of volatile compounds expressed as peak area and relative percent of the total were compared at three levels of oxidation. The average coefficient of variation for peak areas of each volatile ranged from 6.5 to 7.6 for the direct injection method, 5.8 to 6.8 for the dynamic headspace method and 4.5 to 5.4 for the static headspace method. The total area measured by each method increases with oxidation. However, the ratios of the total areas, as they change with peroxide value, are different for each method: 1:1.2:2.8 for direct injection; 1:8.2:33.8 for dynamic headspace, and 1:2.8:3.3 for static headspace.

Most volatile compounds measured by peak areas increase with PV by all three methods. Pentane increases in intensity at all PV's (from 1.7 to 9.5) as measured by the direct injection and by the dynamic headspace GC methods. By the static headspace method, pentane increases from PV 1.7 to 4.9, but does not change between PV 4.9 and 9.5. Pentanal and hexanal increase with PV by the dynamic and the static headspace methods. However, pentanal increases while hexanal decreases by direct injection between PV from 4.9 to 9.5. 2-Heptenal decreases between PV 1.7 and 4.9 by the direct injection method but does not change by the static headspace method. *trans*-2,*trans*-4-Heptadienal increases with PV by all methods. *cis*-2,*trans*-4-Heptadienal decreases between PV from 1.7 to 4.9 by the direct injection and increases with PV by the dynamic headspace and the static headspace methods. Both isomers of decadienal increase with PV by all three collection methods, but the increase is not significant by the static headspace method.

The relative percent volatile composition is changed according to the GC method used. The static headspace method favors the relative proportion of low molecular weight compounds, such as acrolein, propanal and pentanal. Because of the higher vapor pressure of these compounds, they appear in the greatest proportion in the equilibrium mixture as they are eluted on the GC column (15). Pentane is an important volatile compound determined by the direct injection and static headspace methods. The relative concentrations of hexanal and 2-heptenal are high by the static and dynamic headspace methods and lower by the direct injection method. 2,4-Heptadienal is relatively more important by direct injection and dynamic headspace than by static headspace. When analyzed by either direct injection or dynamic headspace, *trans*-2,*trans*-4-heptadienal is larger than the *trans,cis*-isomer. 2,4-Decadienal is the major compound determined by both direct injection and dynamic headspace. At PV of 9.5, the relative percent of both decadienal isomers increases to 45.8% of the total volatile peak area by the direct injection method and to 51.6% by the dynamic headspace method. Most of the minor constituents are not affected by the type of method used, and little difference is found among the methods tested.

The possible origins of each compound are listed in the last column of Table 1. Studies on the decomposition of pure hydroperoxides show that pentane, pentanal and hexanal originate from the 13-hydroperoxide of linoleate (12,13). These volatile compounds dominate by the static headspace analysis. 2-Heptenal, tentatively reported as being formed by the decomposition of 12-linoleate hydroperoxide, is shown as an important compound by all three methods. 2,4-Heptadienal formed from the 12-linolenate hydroperoxide isomer is found in the greatest relative proportion by dynamic headspace analysis, followed by the direct injection method. Decadienal originates from the decomposition of the 9-linoleate hydroperoxide isomer and is the major component measured by the direct injection and dynamic headspace methods. Volatile compounds formed from the decomposition of oleate hydroperoxides, such as octanal, nonanal and 2-decenal (12–14), are much smaller in intensity, and little change occurs with PV by all methods examined.

Advantages and disadvantages are apparent with each GC method for volatile analysis. For routine analyses, static headspace GC is the method of choice because it is rapid and requires no cleaning between samples. GC by direct injection is also rapid and suitable for routine analyses, but an extra step is needed to clean the injector liner between each sample. Dynamic headspace is a much slower method because the sample tube and trapping column are difficult to clean, and often require purging of the system to completely remove some volatile compounds. Both direct injection and static headspace GC require higher temperatures (usually about 150 C), and the volatile analyses include decomposition of flavor precursors. By operating at lower temperatures (as low as 60 C), dynamic headspace may measure actual oxidation products present in oils without thermal decomposition of flavor precursors (9). Another advantage is that it permits component enhancement to reveal minor components that may have flavor significance. A judicious use of several GC techniques is necessary to evaluate volatiles both at the time of tasting and volatiles generated during analysis from lipid oxidation precursors.

REFERENCES

1. Waltking, A.E., and A.G. Goetz, *CRC Crit. Rev. in Food Sci. and Nutr. 19*:99 (1983).
2. Evans, C.D., G.R. List, R.L. Hoffman and H.A. Moser, *J. Am. Oil Chem. Soc. 46*:501 (1969).
3. Selke, E., H.A. Moser and W.K. Rohwedder, *Ibid. 47*:10 (1970).
4. Dupuy, H.P., S.P. Fore and L.A. Goldblatt, *Ibid. 48*:876 (1971).
5. Hartman, K.T., L.C. Rose and R.L. Vandaveer, *Ibid. 48*:178 (1971).
6. Pattee, H.E., E.O. Beasley and J.A. Singleton, *J. Food Sci. 34*:388 (1985).
7. Snyder, J.M., E.N. Frankel and E. Selke, *J. Am. Oil Chem. Soc. 62*:1675 (1985).
8. Warner, K., and E.N. Frankel, *Ibid. 62*:100 (1985).
9. Selke, E., and E.N. Frankel, *Ibid. 64*:749 (1987).
10. Mordret, F., O. Morin and J.-L. Coustille, *Rev. Fr. Corps Gras 32*:193 (1985).
11. *Official Methods and Recommended Practices of the American Oil Chemists' Society,* 3rd edn., edited by R.O. Walker, AOCS, Champaign, IL, Method Cd 8-53.
12. Frankel, E.N., in *Flavor Chemistry of Fats and Oils,* edited by D.B. Min and T.H. Smouse, American Oil Chemists' Society, Champaign, IL, 1985, p. 1.
13. Frankel, E.N., W.E. Neff and E. Selke, *Lipids 16*:279 (1981).
14. Selke, E., E.N. Frankel and W.E. Neff, *Ibid. 13*:511 (1978).
15. Ioffe, B.V., and A.G. Vitenberg, *Headspace Analysis and Related Methods in Gas Chromatography,* John Wiley & Sons, New York, 1984.

[Received November 20, 1987;
accepted April 15, 1988]