# Tandem Gas Chromatography-Mass Spectrometry Analysis of Volatiles From Soybean Oil[1]

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## Abstract

A time vs. volatile decomposition study was made on commercially available soybean oil aged under normal laboratory room conditions. Samples were taken at weekly intervals for peroxide value (PV) determinations, organoleptic evaluations and volatile analyses. Volatiles, stripped from the oil samples in an all-glass system devoid of connecting joints, were collected and sealed in attached capillary tubes. The encapsulated compounds were introduced into a tandem gas chromatograph-mass spectrometer via a capillary crusher. All collections, transfers or introductions of volatiles were performed without the aid of solvents. Hydrocarbons and, subsequently, aldehydes were the principle volatiles associated with the initial and intermediate stages of soybean oil autoxidation (PV 0–11). A variety of other compounds were also found as the level of autoxidation increased. Identified compounds, PV's and organoleptic evaluations are correlated.

## Introduction

Autoxidation of soybean oil and the resulting formation of odors and flavors have been investigated for many years. In early work, Schepartz and Daubert (1) and Kawahara and Dutton (2) used dinitrophenylhydrazine derivatives in their study of volatile compounds; however, this method of analysis is limited to carbonyl-containing compounds. With the advent of gas chromatography (GC) and the development of refined collection techniques, the possibility of identifying volatiles was broadened to include essentially all classes of compounds.

Unfortunately, GC has its limitations as an identification technique, especially when unknown compounds are involved. To circumvent the problem, Smouse and Chang (3) and Chang et al. (4) employed IR and mass spectrometry (MS) to help characterize various isolated soybean oil volatiles. The advantages of GC in separating mixtures of compounds and of MS in identifying separated compounds are evident. To our knowledge, the tandem arrangement of GC and MS techniques and the inherent increase in analytical power have not been applied to the separation and identification of volatiles from soybean oil.

The object of this work was to identify the volatiles formed during the storage of soybean oil, on a time vs. volatile product basis, and to collate these products with organoleptic evaluations and chemical indexes of stability. To facilitate the study a vacuum distillation and collection system was designed to utilize good vacuum techniques and to minimize contamination. In principle this system is similar to that of DeBruyn and Schogt's (5).

## Experimental Procedures

The soybean oil used was a refined, bleached and deodorized commercial salad oil, which was stored at −18 C until needed. Eight liters of the oil was put in a narrow-neck, 9 liter bottle then sealed with a rubber stopper wrapped in aluminum foil. The oil was aged at laboratory temperatures (ca. 72 F) without protection from the room's fluorescent lights. Each day the 9 liter bottle was shaken in order to mix the oil and the head-space air. Each week a 600 ml sample of the aged oil was withdrawn for analysis. When sampling, no precautions were taken to prevent air from entering the bottle. Aliquots were taken from each sample for initial peroxide value (PV) determinations and organoleptic evaluations (6). The remainder of the withdrawn sample was stored under nitrogen at 0 C until vacuum stripped. PV's were again determined just before and immediately after stripping to monitor changes that might have taken place in the oil. The oils were also evaluated by the taste panel to determine the effect of stripping.

The all glass apparatus shown in Figure 1 (E to I) was free from grease, gaskets or interconnecting joints. The apparatus was designed so that the empty system could be evacuated and heated with a flame to remove any residual volatiles and water from the surface of the glass system (baked out). Also, Flask I containing the oil was discarded after each complete stripping procedure to prevent contamination of succeeding samples. The two capillary tubes F and F′ were drawn out from the larger tubing with a gas-oxygen flame. Before stripping, Flask I, which would later contain the soybean oil sample, had its neck extended and stoppered as indicated by the dashed line in Figure 1.

From 70 to 130 ml of aged soybean oil was put into Flask I via a funnel. None of the sample was allowed to come in contact with the upper portions of the flask. The neck was then heated with a gas-oxygen torch, pulled off and sealed at N (Fig. 1).

Vacuum stripping of volatiles from the original and aged soybean oil was performed in two steps. The first step commences with the collection of volatiles in the liquid nitrogen-cooled spiral trap (G) while the charge of oil was degassed at room temperature. The degassing pumping rate was never allowed to exceed 3 mm/min and was regulated by valve E; during this interim, valve H was always open. When the system achieved a constant minimum pressure, as indicated by the vacuum gauge (B), both Teflon valves were closed. Volatiles that condensed in the spiral trap were then transferred into one of the capillary tubes (F or F′) by removing the liquid nitrogen (L) from around the condensing trap (G) and slowly raising the liquid nitrogen (M) around one of the capillary tubes. The partially filled capillary tube F′ was then heated just above the liquid nitrogen level with a gas-oxygen torch, which simultaneously sealed off the capillary tube and sealed its connecting position in the system without breaking vacuum.

The second step in the distillation procedure commenced while the entire system, including the collection section, was still under vacuum from the degassing process. The spiral trap (G) was again cooled

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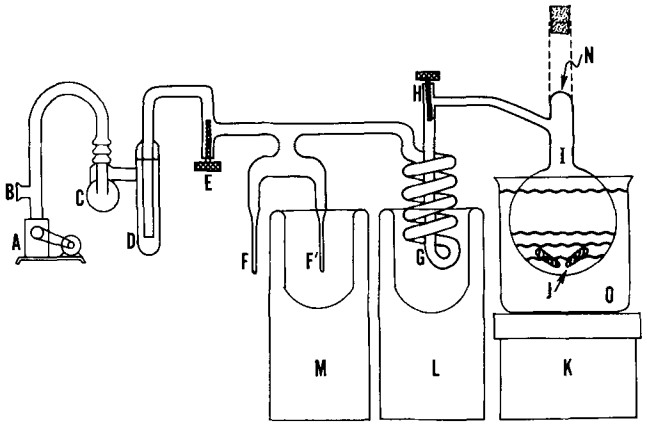


FIG. 1. Vacuum distillation and collection system: A, vacuum pump; B, vacuum gauge; C, diffusion pump; D, liquid nitrogen isolation trap; E, Teflon valve; F and F', two capillary transfer tubes; G, volatile condensation trap; H, Teflon valve; I, 1000 ml round-bottomed flask; J, two Teflon coated stirring bars; K, combined hot plate and magnetic stirrer; L and M, liquid nitrogen dewares; N, flame sealed neck of 1000 ml flask; and O, oil bath.

with liquid nitrogen, the oil bath (O) surrounding the 1000 ml flask was heated to about 90 C, the soybean oil agitation (K) was increased and the oil diffusion pump was turned on. After the newly desired distillation conditions were reached, the two Teflon valves were opened. The collection of vacuum-stripped volatiles then proceeded for 5 to 6 hr. Again, termination of stripping, transfer of condensed volatiles and sealing and removal of the second capillary tube were accomplished as previously described. Only those volatiles collected from the second step in the vacuum-distillation procedure were analyzed. Changes in composition were minor between volatiles collected in steps one and two; however, step two produced the larger quantity of volatiles.

The apparatus was evaluated for its collection efficiency by running three recoveries of a test mixture. The test mixture consisting of methyl esters of normal chain $C_6$ to $C_{18}$ fatty acids was stripped from 100 ml of bland soybean oil. Collection of the test mixture, about 1 ppm of each ester, from the oil was in the manner described except both collection steps were combined into a single distillation at the prescribed temperature (Table I). Recovery percentages, shown in Table I, were calculated by comparing the integrated gas liquid chromatographic (GLC) peak areas of the recovered methyl esters to those of the $C_6$ to $C_{18}$ methyl esters mixture injected directly into the gas chromatograph. This method of obtaining data for recovery calculations is very similar to that used by Angelini (7). The data from three recovery tests in Table I show that the percentage recoveries depend both on the distillation temperature and upon the boiling point of each compound. Our collection efficiences compared favorably with those reported by Angelini et al. (7) and by Forss and Holloway (8) who used the vacuum-distillation method for collecting 10 and 1 ppm, respectively, of $C_3$ to $C_{12}$ methyl ketones from butter oil. In addition to efficiency evaluations, periodic blanks were run on the empty system to guarantee that the glass system was clean and therefore would not contaminate the collected volatiles.

A model SI-4 F&M capillary crusher was used to introduce the collected soybean oil volatiles into the GC-MS system. This GC accessory enabled encapsulated material to be introduced into the GC-MS system without any contaminating solvents. The volatiles were separated on a temperature-programmed (40–275 C) 40 in. × ¼ in. aluminum column packed with 20% poly(ester-acetals) (9) on Chromosorb W. The GC exit port was connected to the MS via a Watson-Biemann helium separator (10). Mass spectra were simultaneously photographed and chart recorded from a Bendix Model 12 time-of-flight mass spectrometer. The mass spectra were scanned from M/e 12–200 in 11 sec.

TABLE I

Recovery of Added $C_6$-$C_{18}$ Methyl Esters From 100 Ml of Soybean Oil (SBO)

| Methyl ester | BP, C | Concentrations in 100 ml SBO, ppm | Recovery,[a] % Distillation temp., C | | |
|---|---|---|---|---|---|
| | | | 93 | 92 | 72 |
| Hexa- | 151 | 0.075 | 90 | 122 | 94 |
| Hepta- | 174 | 0.38 | 22 | 38 | 34 |
| Octa- | 193 | 0.92 | 53 | 23 | 22 |
| Nona- | 213 | 1.01 | 47 | 30 | 12 |
| Deca- | 224 | 1.27 | 35 | 40 | 14 |
| Undeca- | 250 | 1.58 | 13 | 11 | 6 |
| Dodeca- | 268 | 1.39 | 5 | 3 | 0.8 |
| Trideca- | 286 | 1.39 | 1 | 3 | 0.8 |
| Tetradeca- | 300 | 1.05 | 0.3 | Trace | Trace |

[a] No $C_{15}$-$C_{18}$ methyl esters were recovered from SBO.

## Results and Discussion

To initiate our study, soybean oil was aged at room temperature and sampled at weekly intervals to obtain organoleptic scores, peroxide values and predominant flavor descriptions (Table II). The PV's indicated that a slow rate of autoxidation occurred during the first four weeks of storage, similar to an induction period, and that then the rate increased until there was almost a linear relationship between time and PV's. The last two columns in Table II show the change in flavor scores and predominant flavors with the degree of autoxidation. The flavor, the more predominant listed first, changed from a slightly buttery-nutty taste (PV = 0.4) to a beginning rancid stage (PV = 42) and reflected the normal transition of a nearly bland to an autoxidized oil.

All the samples were vacuum stripped at ca. 90 C, except the oil aged five, six and eight weeks. These three samples were used to determine the optimum, vacuum-stripping temperature; i.e., to produce the maximum amount of volatiles without perceptible hydroperoxide decomposition. Table III contains the results when stripping temperatures of 62, 90 and 125 C were investigated. When a temperature of 62 C was used, the PV remained essentially the same after stripping as before (note data for the oil aged eight weeks). However, the total amount of material collected was less than an estimated 5 μl compared to

TABLE II

Evaluations of Aged Soybean Oil

| Storage,[a] weeks | PV[b] meq/kg | Flavor | |
|---|---|---|---|
| | | Score[c] | Taste description[d] |
| 0 | 0.4 | 6.6 | Buttery, nutty |
| 1 | 0.8 | 6.2 | Buttery, beany |
| 2 | 1.1 | 5.6 | Buttery, grassy |
| 3 | 2.2 | 4.8 | Buttery, grassy |
| 4 | 4.6 | 5.5 | Buttery, rancid |
| 5 | 7.0 | 5.8 | Buttery, rancid |
| 6 | 11 | 4.8 | Buttery, grassy |
| 7 | 18 | 4.7 | Grassy, buttery |
| 8 | 15 | 4.7 | Grassy, rancid |
| 9 | 19 | 5.1 | Grassy, buttery |
| 13 | 42 | 4.1 | Grassy, rancid |

[a] At room temperature.
[b] PV, peroxide value.
[c] On the basis of a 0–10 scale.
[d] The first taste listed was the predominant flavor.

TABLE III

Characteristics of Aged Soybean Oil Before and After Vacuum Stripping

| Aged[a] weeks | PV, meq/kg | | Flavor scores | |
|---|---|---|---|---|
| | Aged oil | Stripped oil | Aged oil | Stripped oil |
| 5 | 7 | 0.0(125)[b] | 5.8 | 2.8 |
| 6 | 11 | 0.4(125) | 4.8 | 3.2 |
| 7 | 18 | 9 ( 97) | 4.7 | 2.9 |
| 8 | 15 | 16 ( 62) | 4.7 | 3.6 |
| 9 | 19 | 20 ( 90) | 5.1 | 2.6 |
| 13 | 42 | 42 ( 91) | 4.1 | 2.9 |

[a] At room temperature.
[b] Figures in parentheses indicate temperatures (C) at which the aged soybean oil samples were vacuum stripped.

more than 20 $\mu$l from the oil aged seven and nine weeks. Also, the sample size ($<5$ $\mu$l) led to unsatisfactory GC-MS analysis because of the small GC peaks and the corresponding weak and unidentifiable mass spectra. Soybean oils aged for five and six weeks were stripped at 125 C. Since the PV's of these stripped oils were essentially zero, the higher stripping temperatures (125 C) decomposed the hydroperoxides. When stripping temperatures of 90 C were investigated (9- and 13-week-aged oils, Table III), the PV's remained the same before and after stripping. Oil aged for seven weeks did show a PV decrease after 97 C stripping; however, it is believed a resulting hot spot (i.e., the 1000 ml flask touching the bottom of the oil bath beaker) caused the PV decrease and not the 97 C oil bath temperature. These analyses showed that vacuum-stripping temperatures in the 90 C region yielded the most volatiles without decomposing hydroperoxides.

The flavor scores listed in Table III show that vacuum-stripping does not produce a bland oil. Organoleptic responses associated with these scores indicate buttery, beany and grassy flavors, reported for the aged oil, diminish or disappear after distillation and that rancid, painty, fishy and cucumber flavors either are produced or are intensified. Thus, vacuum-stripping produces a less desirable oil in which harsher flavors have been enhanced. This phenomenon is not unusual. Hill and Hammond (11) report that in their rota-film distillation at a temperature of 70 C their stripped soybean oil developed a slightly fishy flavor, and at 170 C fishy and heated oil flavors actually became more intense. Thus, changes in flavor descriptions and scores point to the possibility that the earlier developed flavors mask those developed during later stages of autoxidation.

Figures 2 and 3 are GC curves of volatiles stripped from soybean oil aged for four and nine weeks. The curve in Figure 2 represents chromatograms of volatiles during early stages of autoxidation or the induction period. Water, pentane and carbon dioxide are the predominant peaks; other components cause just discernible peaks. Compounds associated with these small peaks are essentially hydrocarbons (12), and at this autoxidation level they represented about one-half of the identified compounds. Four normal aldehydes ($C_2$, $C_3$, $C_5$ and $C_6$ correspond to GC peaks numbers 5, 6, 12 and 14, respectively, in Fig. 2) are present in minor quantities; however, they become more abundant at higher autoxidation levels.

Figure 3 is a GC curve of volatiles stripped from soybean oil aged for nine weeks and is representative of chromatograms produced from volatiles associated with later autoxidation stages; i.e., when oxygen-containing compounds predominate. With the exception of three compounds, acetic acid and those with molecular weights of 126 and 166, all other compounds

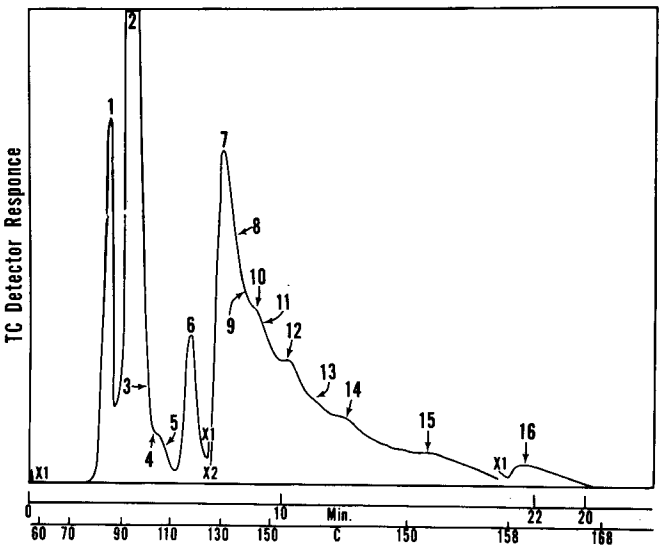


FIG. 2. Gas chromatogram of volatiles from four-week-old soybean oil (PV 4.6 meq/kg). 1, Carbon dioxide, ethane, propane; 2, Pentane; 3, 2-Pentane; 4, Hexane; 5, 1-Hexene, ethanal; 6, Propanal; 7, Water; 8, Ethanol; 9, Octane; 10, 2-Octene; 11, MW = 110 (?-Dienal, substituted furan or alkyldiene); 12, Pentanal; 13, 2-Butenal; 14, Hexanal; 15, MW = 124. Substituted furan, tentative; 16, 4,5-Octadione, tentative.

listed in Figure 3 appear in volatiles stripped from soybean oil aged five or more weeks.

The unidentified compound partially responsible for GLC peak 11 in Figure 2 and 10 in Figure 3 has mass fragment peaks of pentanal and 2-octene included in its spectrum. Nevertheless, the parent ion at M/e 110 (ca. 30% of the base peak—M/e 81) is distinguishable and is in agreement with an elemental composition of $C_7H_{10}O$ (14). Major mass peaks unique to this compound are at 39, 53, 55 and 110 with minor ones at M/e 29, 67, 68 and 95. These mass fragment ions are characteristic of dienals (D. R. Black, unpublished), substituted furans (4,13–15) and alkyl dienes (18). Much of the spectral pattern fits that of dienals and furans, though a few other mass peaks; e.g., M/e 55 (ca. 80% of the base peak), are more closely associated with various types of hydrocarbon compounds.

A compound only tentatively identified is associated with GLC peak 15 in Figure 2 and 16 in Figure 3. It has a mass spectral pattern (M/e 29, 39, 53, 55 and 81) resembling that of the unidentified compound producing GLC peaks 11 and 10 also in Figures 2 and 3, respectively. This compound has a clearly discernible molecular ion at M/e 124 (ca. 30% of the base peak—M/e 81) which may correspond to a molecular formula of $C_8H_{12}O$ (17). The most intense ions are the base peak, the molecular ion and M/e 79, contributing, respectively, 25%, 7% and 7% to the total ion current. These plus the other mass fragment ions are common to dienals and furans. Reportedly the mass spectra of some alkyl furans and dienals are nearly identical when obtained under GC-MS fast scan conditions (16). Therefore one method to distinguish the spectra of the two types of compounds depends on noting subtle differences in ratios and percentage total ionization of the various ions in their spectra; e.g., M/e 53, 55 and 67, plus those ions in the M/e 81 region (D. R. Black, unpublished). When this method is followed in analyzing the mass spectrum, the MW-124 compound is an alkyl furan rather than a dienal. However, no matching spectrum could be found in

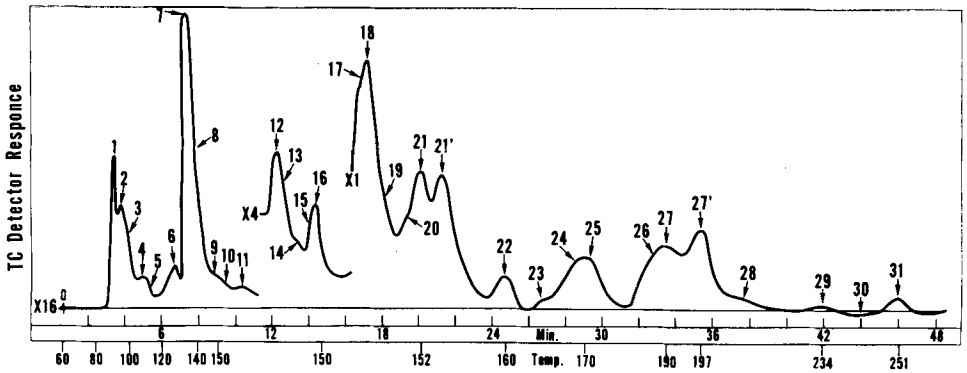


FIG. 3. Gas chromatogram of volatiles from nine-week old soybean oil (PV 23 meq/kg). 1, Carbon dioxide, ethane, propane, butane; 2, Pentane; 3, 2-Pentene, diethyl ether; 4, Hexane, ethanal; 5, 1-Hexene; 6, Propanal, carbon disulfide; 7, Water, methanol; 8, Ethanol; 9, Octane, chloroform, benzene; 10, 2-Octene; MW = 110 (?-Dienal, substituted furan or alkyldiene); 11, Pentanal, toluene; 12, Hexanal; 13, 2-Butenal; 15, 3-Hexenal; 16, MW = 124. Substituted furan, tentative; 17, 4,5-Octadione, tentative; 18, Heptenal; 19, Acetic acid; 21 and 21', 2,4-Heptadienal; 24, MW $\geq$ 142; 25, MW $\geq$ 126; 26, Butyric acid; 27 and 27', 2,4-Decadienal; 31, Cyclic hydrocarbon (MW = 166). Peaks 14, 22, 23, 28, 29 and 30 were not identified. Peaks 21' and 27' are apparent geometrical isomers of 21 and 27, respectively.

our data files; among them being Interscience's Atlas of Mass Spectral Data.

Mass spectra of GLC peaks 16 and 17 in Figures 2 and 3, respectively, show them to be due to the same compound. The material presumably is a ketone and has a parent peak at M/e 142 (ca. 3% of the base peak—M/e 43). This molecular ion can correspond to a molecular formula of $C_8H_{14}O_2$ (17). All major mass fragment ions (M/e 27, 29, 39, 41, 71 and 99) are 20-30% of the base peak. These ions and intensities plus those minor ones at M/e 55, 57, 83 and 85 are typical of aliphatic *n*-propyl ketones (19). Beynon et al. (20) report that alpha cleavage to the carbonyl is an important fragmentation in aliphatic ketones and should produce two major mass peaks, one at P-$R_1$ and the other 28 mass units lower at P-C = $OR_1$. Since there are no fragment ions with intensities greater than 3% of the base peak between the molecular ion-M/e 142 and M/e 99, one of the alkyl groups in this ketone is a $C_3H_7$ moiety. The next lower major fragment is at M/e 71 with the difference (M/e 99–71) showing a loss undoubtedly of a carbonyl. These observations are in agreement with the alpha cleavage fragmentation reported y Beynon et al. (20). Because there are no high intensity ions in the M/e 57 and 85 mass regions and because M/e 43 is the next lower major ion (loss of 28 mass units from fragment ion 71), apparently there are two adjacent carbonyl groups in this compound. Although this compound can be more or less identified as a dicarbonyl ketone, it may be 4,5-octadione; however, the mass spectrum of this ketone has similarities to that published for authentic 2,3-octadione (21).

Mass spectra of compounds from GLC peaks 24 and 25 in Figure 3 were weak and included fragment ion peaks of adjacent compounds. At the most, only molecular weight (MW of 142 from GLC peak 24 and MW of 126 from GLC peak 25) could be gleaned from their mass spectral pattern.

The material (GC peak 31, Fig. 3) with a molecular weight of 166 eluded identification even though a strong spectrum was obtained for analysis. Its spectrum consisted essentially of only two M/e peaks; one at M/e 166 the apparent molecular ion, the other at M/e 137 the base peak. Because of the intense M/e 166 peak the compound is believed to be cyclic and the difference between M/e 166 and M/e 137 peaks suggests it is substituted with a carbonyl or ethyl group.

Two compounds identified, but not found in the aged four- and nine-week oil (Fig.s 2 or 3), were pentadecane and heptadecyne. These two components accompanied volatiles obtained only from the five-, six- and seven-week-old soybean samples. Since the PV's in these samples decreased after being vacuum distilled at high temperatures (Table III), the two compounds are most likely associated with the thermally decomposed hydroperoxides.

In Figure 3, the identified compounds, chloroform and carbon disulfide, were probably absorbed by the SBO from laboratory air; however, the origin of toluene, benzene, methanol and ethanol could be either from room air or secondary autoxidation products.

Authentic compounds of hydrocarbons ($C_2$ to $C_8$) and aldehydes ($C_2$ to $C_6$) provided a basis for a quantitative estimation of these components in the volatiles. Initially, hydrocarbons in the $C_2$ to $C_8$ range (Table IV) were the only volatiles collected from the soybean oil samples. Propane and butane occurred in trace quantities and were excluded from the calculations. As shown in Table IV, pentane (91%) and pentene (9%) were the only volatiles obtained from the control oil. Ethane (15%), pentane (77%) and pentene (8%) were obtained from

TABLE IV

Some Volatiles From Soybean Oil

| Aged oils, weeks | Relative per cent | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hydrocarbons | | | | | | | Aldehydes | | | |
| | $C_2$ | $C_5$ | $C_5^{\Delta 2}$ | $C_6$ | $C_6^{\Delta 1}$ | $C_8$ | $C_8^{\Delta 2}$ | $C_2$ | $C_3$ | $C_5$ | $C_6$ |
| 0 | | 91 | 9 | | | | | | | | |
| 1 | 15 | 77 | 8 | | | | | | | | |
| 2 | 17 | 57 | 5 | 1 | | | | 15 | | | 6 |
| 3 | 8 | 53 | 3 | 1 | 0.2 | 1 | 2 | 17 | 8.6 | 3.9 | 2.9 |
| 4 | 8 | 50 | 5 | 1 | 0.1 | 0.8 | 3.4 | 8 | 17 | 4.6 | 2.9 |
| 6 | 3.6 | 21.6 | 1.6 | 0.9 | 0.1 | 0.3 | 1.1 | 7.6 | 41.5 | 9.0 | 12.8 |

oil aged for one week. When the PV of the oil reached one as in the two-week-old oil, only then did some oxygen-containing compounds appear, mainly as ethanal (15%) and hexanal (6%). Data from the two-, three- and four-week-old soybean oil showed that the four aldehydes ($C_2$, $C_3$, $C_5$ and $C_6$) represented 21% to 33% of the 11 compounds listed. However, the combined percentages of these aldehydes increased until the sixth week of autoxidation. From then on, the four aldehydes always accounted for 70% of the 11 components. Also, from the sixth week of soybean autoxidation, the relative percentages of these 11 volatiles remained essentially constant in the proportions shown (Table IV), but their absolute amounts increased with each week of autoxidation.

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REFERENCES

1. Schepartz, A. I., and B. F. Daubert, JAOCS *27*, 367 (1950).
2. Kawahara, F. K., and H. J. Dutton, Ibid. *29*, 372 (1952).
3. Smouse, T. H., and S. S. Chang, Ibid. *44*, 509 (1967).
4. Chang, S. S., T. H. Smouse, R. G. Krishnanmurthy, B. D., Mookherjee and R. B. Reddy, Ibid. *1966*, 1926.
5. DeBruyn, J., and J. C. M. Schogt, JAOCS *38*, 40 (1961).
6. Moser, H. A., H. J. Dutton, C. D. Evans and J. C. Cowan, Food Technol. U.S.A. *4*, 105 (1950).
7. Angelini, P., D. A. Forss, M. L. Bazinet and C. Merritt, Jr., JAOCS *44*, 26 (1967).
8. Forss, D. A., and G. L. Holloway, Ibid. *44*, 572 (1967).
9. Moore, D. J., and V. L. Davison, Abstr. 24, AOCS 41st Fall Meeting, October 1967.
10. Watson, J. T., and K. Biemann, Anal. Chem. *37*, 844 (1965).
11. Hill, F. D., and E. G. Hammond, JAOCS *42*, 1148 (1965).
12. Evans, C. D., Proc. Flavor Chem. Symp., Campbell Soup Company, 1961.
13. Budzikiewicy, H., C. Djerrassi and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day Inc., San Francisco, 1967, p. 615.
14. Grigg, R., M. V. Sargent and D. H. Williams, Tetrahedron *21*, 3441 (1965).
15. Heyns, R. S., and H. Scharmann, Ibid. *22*, 2223 (1966).
16. Black, D. R., M. Nonaka and W. H. McFadden, Abstr. 135, 15th Annual Conference on Mass Spectrometry and Allied Topics, May 1967.
17. Beynon, J. H., "Mass Spectrometry and Its Application to Organic Chemistry," Elsevier Publishing Company, New York, 1960.
18. McLafferty, F. W., "Mass Spectral Correlations," American Chemical Society, Washington, D. C., 1963.
19. Sharkey, A. G., Jr., J. L. Schultz and R. A. Friedel, Anal. Chem. *28*, 934 (1956).
20. Beynon, J. H., R. A. Saunders and A. E. Williams, "The Mass Spectra of Organic Molecules," Elsevier Publishing Company, New York, 1968, p. 190.
21. Stark, W., and D. A. Forss, J. Dairy Res. *29*, 173 (1962).

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