Determination of the Δ15 Double Bond in Fatty Acids of Hydrogenated Soybean Oil[1]

L. T. BLACK and R. E. BEAL, Northern Regional Research Laboratory,[2] Peoria, Illinois 61604

Abstract

A method was developed to determine the extent of hydrogenation of the Δ15 double bond which occurs during partial catalytic hydrogenation of soybean oil. A linear relationship was found to exist between the linolenate content of commonly occurring C_{18} unhydrogenated oils (containing no tetraene) and the propanal resulting from their ozonization reduction. The amount of propanal so produced is directly related to the amount of Δ15 double bond in these oils, as well as in hydrogenated soybean oils. Soybean oil was treated with ozone in carbon tetrachloride at −20 C and then reduced with triphenylphosphine. The ozonized-reduced sample was injected into a gas chromatograph, operated at 170 C and equipped with a 12 ft × ¼ in. column of 100/120 mesh porous polymer beads. The propanal peak was identified and its area used as a measure of the fatty acids containing Δ15 double bonds in unhydrogenated soybean and other oils of known linolenate content. A nearly stoichiometric amount of propanal results from ozonizing, reducing and chromatographing soybean oil as shown by comparison with a standard mixture of propanal and carbon tetrachloride. The relative standard deviation for the method is ±4.4%. We have also found this method applicable to other oils containing the omega-3 double bond.

Introduction

The use of liquid vegetable oils for food products is expanding. However, foods containing those oils with the linolenate fatty acid moiety have been limited because they are more susceptible to flavor reversion and autoxidation than oils in which linolenate is absent (6,7). Selective hydrogenation successfully reduces linolenate content of soybean oils without increasing the saturates (9–11).

During our studies on the selective hydrogenation of soybean oil with a copper-chromite catalyst, the percentage of fatty acids containing the Δ15 double bond in partially hydrogenated oils needed to be determined accurately.

Several procedures have been reported to determine the double bond position in unsaturated fatty acids. Used widely is periodate-permanganate oxidative fission under the conditions of von Rudloff (15), further modified by Downing and Greene (5). For others, reductive ozonolysis coupled with gas chromatography has made quantitative identification of most double bonds quicker and less tedious (1,4,12–14,17). However, none of these methods have proved satisfactory for determining Δ15 because the propanal derived from an omega-3 double bond is difficult to recover and analyze quantitatively (2). When the method presented here was applied, a quantitative relationship was established between the amount of the C_{18} fatty acids having the Δ15 double bond and the amount of propanal from the ozonization reduction of non-hydrogenated vegetable oils, which contained 9,12,15-octadecatrienoate (linolenate). Also, the amount of propanal after ozonization-reduction of hydrogenated soybean oil samples correlated directly with the Δ15 double bond content of the fatty acids.

Experimental Procedures

From 10 to 15 mg of a vegetable oil containing linolenic acid was weighed accurately into a 15 × 45 mm vial with a plastic screw cap. To each vial was added 1 ml of high-purity ACS-grade carbon tetrachloride. The sample was ozonized for 2 min at approximately −20 C in dry ice-ethanol. The ozone was generated by a semimicro ozonizer reported by Bonner (3). The generator produced a high concentration of ozone at an oxygen flow rate of 35 cc/min. Triphenylphosphine (50 mg) was added to reduce the ozonides. This step was followed by addition of a 0.25 ml aliquot of carbon tetrachloride containing a known amount of diethyl ether (0.1–0.2 mg) used as an internal standard. A 5–10 μl sample was injected into an F&M Model 700 gas chromatograph equipped with a flame ionization detector. Operating conditions were as follows: Column temperature 170 C, detector temperature 250 C, injection port ambient and helium flow of 75 cc/min. The sensitivity of the instrument was adjusted so that the propanal derived from an unhydrogenated soybean oil gave 80–90% of recorder scale. The column was 12 ft × ¼ in. and contained 100/120-mesh Porapak Q. Propanal was identified by gas chromatographic retention data from three Porapak columns of different polarity. A blank sample was run the same day; however, at the highest sensitivity only a trace peak appeared at the retention time of propanal. Once a sample had been ozonized and reduced it was immediately injected into the chromatograph. Samples allowed to age before injection lost propanal. Because of the use of an internal standard, direct comparisons of the peak areas representing propanal were unnecessary between different sample injections, initial warm-up and stabilization time for the chromatograph were held to a minimum.

Approximately 45 min were required for the carbon tetrachloride to purge from the column; however, by raising the temperature 75 C for 10 min between injections, this purge was reduced to about 15 min.

Whenever possible, peak areas were determined with a CRS-11 Infotronics digital integrator. Peaks with severely drifting baselines were xeroxed, cut and weighed, however any other convenient way to quantitate the peak area may be employed. The calculation was as follows: $[(P)(W)/(S)]/A = M$: P = Peak area of propanal; W = Mg diethyl ether in ozonized-reduced sample; S = Peak area of diethyl ether; A = Mg sample or unhydrogenated soybean oil (known linolenate); M_1 = Mg propanal/mg sample; M_2 = Mg propanal/mg unhydrogenated soybean oil; $(L)(M_1)/M_2 = X$: L = Linolenate in unhydrogenated soybean oil standard, %; X = C_{18} fatty acids containing Δ15 double bonds, %.

[1] Presented at the AOCS-AACC Meeting, Washington, D.C., March, 1968.
[2] No. Utiliz. Res. Dev. Div., ARS, USDA.

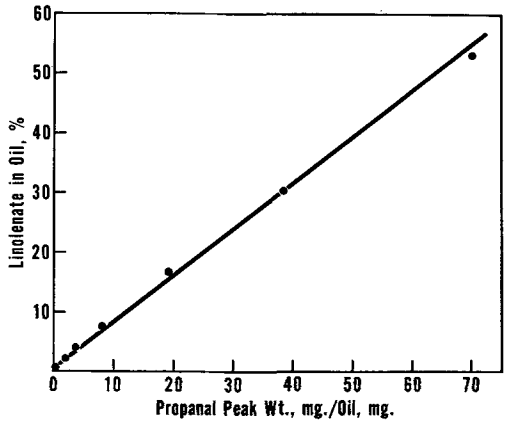

FIG. 1. Relationship between linolenate and propanal derived by reductive ozonolysis.

Results and Discussion

Figure 1 illustrates the linear relationship existing between the linolenate content of nonhydrogenated vegetable oil and the quantity of propanal after reductive ozonolysis. This relationship was demonstrated by mixing corn, soybean and linseed oils of known linolenate content to gain increasing proportions of linolenate and, subsequently, by measuring the propanal liberated by reductive ozonolysis.

On the basis of this relationship any nonhydrogenated soybean oil which contains a known percentage of linolenate (that is, 9,12,15-octadecatrienoate) contains the same percentage of acids with Δ15 double bonds. Consequently, any nonhydrogenated C_{18} vegetable oil that has a known percentage of linolenate may serve as a standard for any C_{18} oil with the omega-3 double bond.

Figure 2 is a chromatogram produced from an ozonized-reduced soybean oil. The first peak is one with an identical retention time to that of acetaldehyde. The next two are a mixture of decomposition peaks from triphenylphosphine. These are followed by the propanal and diethyl ether peaks.

The effect of ozonization time is seen in Figure 3. When identical soybean oil samples were ozonized at increasing time increments, the minimum ozonization required was 2 min as indicated by the amount of propanal measured after reduction. The presence of excess ozone in the effluent gas from the solution did not indicate complete ozonolysis.

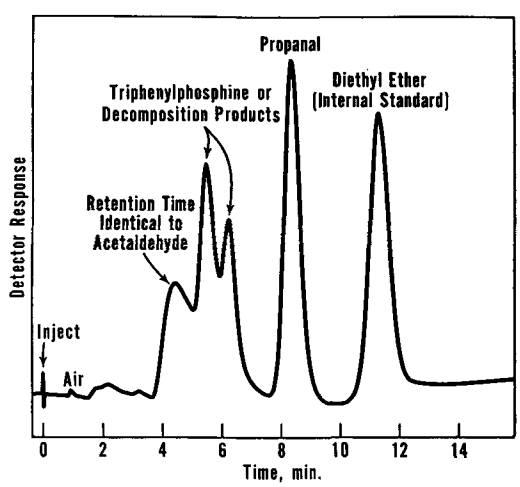

FIG. 2. Ozonized-reduced soybean oil.

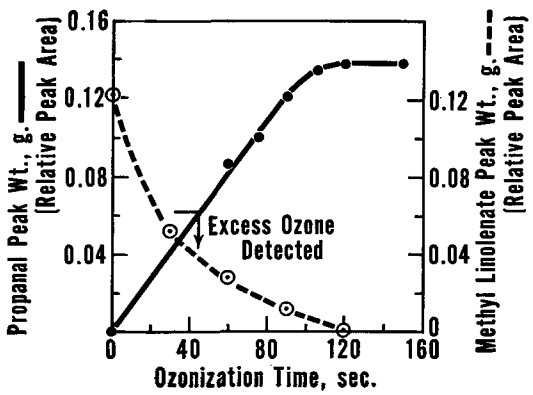

FIG. 3. Effect of ozonization time on triglycerides and methyl esters.

The necessity of a 2 min ozonization was confirmed by ozonizing identical samples of methyl linolenate and subsequently following its disappearance by gas liquid chromatography (Fig. 3). Several identical samples of methyl linolenate in carbon tetrachloride were prepared and ozonized for intervals up to 2 min. Each sample, including one which had not been ozonized, was chromatographed on a silicone column. Unreacted methyl linolenate was present in every sample that was ozonized less than 2 min.

Figure 4 shows the effect when the amounts of triphenylphosphine reducing agent are varied. A 15 mg soybean oil sample requires at least 40 mg of triphenylphosphine to reduce the ozonides completely.

A 15 mg soybean oil sample containing 8.0% linolenate was ozonized and reduced. The propanal peak obtained by chromatographing the sample was compared with that of a standard propanal-CCl_4 mixture. This comparison indicated that the ozonization conditions selected would yield greater than 90% of the stoichiometric amount of propanal from soybean oil.

The precision of the standardization procedure was established from a series of identical soybean oil samples. The propanal from 10 identical samples ozonized and reduced on the same day gave a relative standard deviation of ±2.4%, whereas 6 identical samples from another day showed a ±1.9% relative standard deviation. The precision of the analysis on hydrogenated soybean oils is shown in Table I. Five samples of soybean oil at various levels of hydrogenation were analyzed for linolenate by gas chromatography. These samples were then analyzed for the percentage of Δ15 double bonds in the fatty acids on five different days.

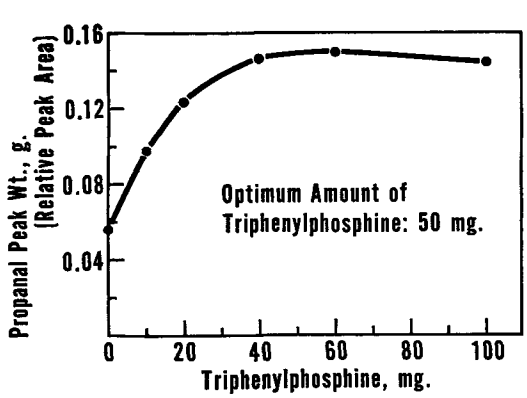

FIG. 4. Effect of varying amounts of triphenylphosphine.

TABLE I

Precision of Δ15 Double Bond Analysis of Hydrogenated Soybean Oil

Methyl linolenate, %	Fatting acids containing Δ15 double bond, %					Relative standard deviation, %
	1st Day	2nd Day	3rd Day	4th Day	5th Day	
5.7	6.8	6.5	6.6	6.9	6.6	±2.8
3.5	5.1	4.8	5.1	4.9	5.2	±3.4
2.7	4.4	4.6	4.8	4.3	4.5	±4.4
1.9	3.9	3.6	3.9	3.4	3.8	±3.7
1.2	3.3	3.2	3.3	3.1	3.5	±3.3

The maximum relative standard deviation was ±4.4%.

When linolenate in the soybean oil reached 1% (as a result of partial hydrogenation), the fatty acids containing Δ15 double bonds were still approximately 3%. This difference can be accounted for by the remaining presence of linolenate + isolinoleate (Δ15 dienes) + isooleate (Δ15 monoene).

Possibly butanal, which is also eluted from Porapak Q, could be used as a measure for omega-4 double bonds.

Chromatographic results from the reductive-ozonolysis of methyl 9,12-octadecadienoate (methyl linoleate) show a peak with the same retention time as acetaldehyde. Acetaldehyde would not be expected from reductive-ozonolysis of methyl linoleate since omega-2 double bonds are not present, but wherever great numbers of methylene-interrupted double bonds occur, large quantities of this unknown peak were found. Since reductive-ozonolysis of a sample containing methylene-interrupted double bonds yields malonaldehyde, perhaps the extremely unstable malonaldehyde forms some acetaldehyde by decarbonylation. Larger quantities of this unknown appeared when the injection port temperature was raised above ambient. Therefore, the evidence indicates that the detection of acetaldehyde does not prove the presence of an omega-2 double bond.

We have successfully applied this method to other common vegetable oils including corn, olive, linseed and cottonseed. In addition, other workers have applied it to oils with acyl groups containing omega-3 double bonds. Some of these oils which include *Ephedra campylopoda* (8) and *Caltha palustris* (16) have uncommon fatty acid structures.

REFERENCES

1. Benton, F. L., A. A. Kiess and H. J. Harwood, JAOCS *36*, 457 (1959).
2. Beroza, M., and B. A. Bierl, Anal. Chem. *39*, 1131 (1967).
3. Bonner, W. A., JAOCS *30*, 452 (1953).
4. Davison, V. L., and H. J. Dutton, Anal. Chem. *38*, 1302 (1966).
5. Downing, D. T., and R. S. Greene, Lipids *3*, 96 (1967).
6. Dutton, H. J., C. R. Lancaster, C. D. Evans and J. C. Cowan, JAOCS *28*, 115 (1951).
7. Evans, C. D., Flavor Chemistry Symposium, Campbell Soup Company (1961).
8. Kleiman, R., G. F. Spencer, F. R. Earle and I. A. Wolff, Chem. Ind. *31*, 1326 (1967).
9. Koritala, S., and H. J. Dutton, JAOCS *43*, 86 (1966).
10. Koritala, S., and H. J. Dutton, Ibid. *43*, 556 (1966).
11. Okkerse, C., A. DeJonge, J. W. E. Coenen and A. Rozendaal, Ibid. *44*, 152 (1967).
12. Privett, O. S., and Christense Nickell, Ibid. *39*, 414 (1962).
13. Privett, O. S., and E. C. Nickell, Ibid. *41*, 72 (1964).
14. Privett, O. S., and E. C. Nickell, Ibid. *43*, 393 (1966).
15. Rudloff, E. von, Can J. Chem. *34*, 1413 (1950).
16. Smith, C. R., Jr., R. Kleiman and I. A. Wolff, Lipids *3*, 37 (1968).
17. Stein, R. A., JAOCS *42*, 326 (1963).

[Received January 13, 1969]