Flavor Reversion in Soybean Oil. V. Isolation and Identification of Reversion Compounds in Hydrogenated Soybean Oil[1,2]

A. I. SCHEPARTZ and B. F. DAUBERT, Department of Chemistry, University of Pittsburgh, Pittsburgh, Pa.

IN a previous communication from this Laboratory (1) evidence was presented that indicated the occurrence of carbonyl materials in reverted, hydrogenated soybean oil, also that these materials contributed to the reverted flavor. 2-Heptenal as well as an unknown dicarbonyl compound were isolated as the 2,4-dinitrophenylhydrazones. Since these experiments were conducted on a relatively small scale, it was felt that perhaps other compounds in addition to the two already found might have been present but were missed because of their low concentrations. Accordingly, a reinvestigation of the deodorization condensates was launched on a scale much greater than had previously been attempted. In addition, the dicarbonyl compound previously isolated (1) was identified.

Experimental

The soybean oil used in this study was a partially hydrogenated product (I.V. 103) supplied by Wilson and Company, Chicago, Illinois.

1. *Collection of Condensates.* The flavor materials present in the reverted oil were removed by steam deodorization for one hour at 200°C. and 1-2 mm. pressure, using the apparatus designed by Bailey and Feuge (2). A quantity of 1,700 ml. of oil was reverted by heating to 200°C., cooled rapidly, and then deodorized. In order to obtain a large number of condensates, this process was repeated 275 times. The oil sample was replaced after every 15 deodorizations. The total volume of oil used was 28.9 liters.

One series of 15 reversions and deodorizations was sampled at intervals and peroxide values determined by the method of Wheeler (3). The average peroxide value after reversion was 1.53 while the average after deodorization was 0.95.

After each day's deodorizations the Dewar traps (2) were washed out with aldehyde-free ethyl ether and the total condensates stored at 6°C. These consisted of two phases, ether and water. The ether layer was a light yellow-green in color; the water layer was almost colorless.

A summary of all of the following separations is given in Figure 1.

2. *Separation of the Ether-Soluble from the Water-Soluble.* After decreasing the volume of the ether layer by evaporation on a water aspirator until equal to that of the water layer (800 ml.), the two phases were separated. The ether phase was washed five times (500 ml.) with water (these water washings were added to the water layer), dried over sodium sulfate, and then filtered. After removal of the ether by aspiration under nitrogen, the ether-soluble fraction remaining (S-167-E) weighed 23.8599 g.

The water phase was saturated with ammonium sulfate and extracted with ether in a separatory funnel. This extract was dried over sodium sulfate, filtered, and the ether removed as above. The weight of this water-soluble fraction (S-168-W) was 2.0695 g.

3. *Vacuum Distillation at Room Temperature.* A quantity of 23.1436 g. of the ether-soluble fraction (S-167-E) was distilled at room temperature and a pressure of 0.6 mm., with agitation provided by a magnetic stirrer. Since it had been shown earlier (1, 4) that the flavor materials were quite volatile, a vacuum distillation procedure was adopted as a means of further concentration. The material which was volatile under these conditions was condensed in a spiral trap immersed in a dry-ice-acetone bath. After a total of 32 hours the rate of distillation was too slow to be significant, and the process was halted. The weight of ether-soluble volatile material (S-169-EV) obtained was 2.6493 g. The residue in the flask (S-167-ER) weighed 19.9288 g.

The obvious discrepancy in the weights given above is typical of all weighings involving the volatile fractions. Their high volatility made it exceedingly difficult to remove all of the ether without loss of material.

The water-soluble fraction (S-168-W) was distilled under the same conditions as above, using 1.8627 g. of material. After 28 hours the yield of water-soluble-volatile fraction (S-170-WV) was 0.4691 g. The residue remaining (S-168-WR) weighed 1.2895 g.

4. *Absorption Spectra of Fractions.* Weighed samples of each of the above six fractions were removed and dissolved in ethanol. The ultraviolet absorption spectra of these solutions were determined on a Beckman Model DU quartz spectrophotometer. The results of plotting the specific extinction value against the wavelength are presented in Figures 2 and 3.

5. *Hydrogenation Studies on Fractions.* The apparatus used was similar to that of Noller and Barusch (5) except for the incorporation of a Precision Mine Air Buret, which was graduated in 0.2 ml. from 0 to 72 ml., in 0.1 ml. from 72 to 94 ml., and in 0.05 ml. from 94 to 100 ml. The latter region of the buret enabled easy reading of the small volumes needed in

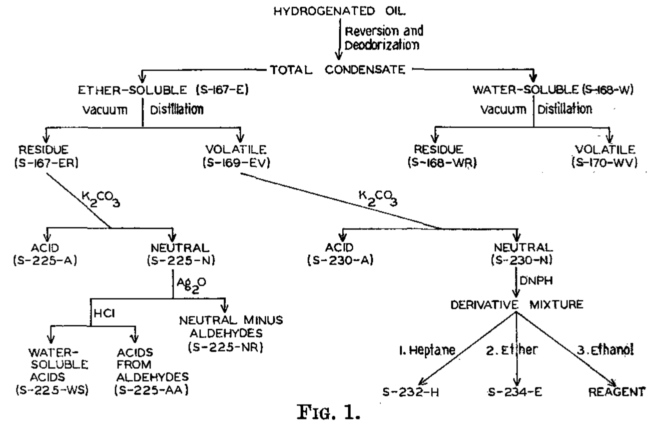

FIG. 1.

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[2] A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. The contract is being supervised by the Northern Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry.

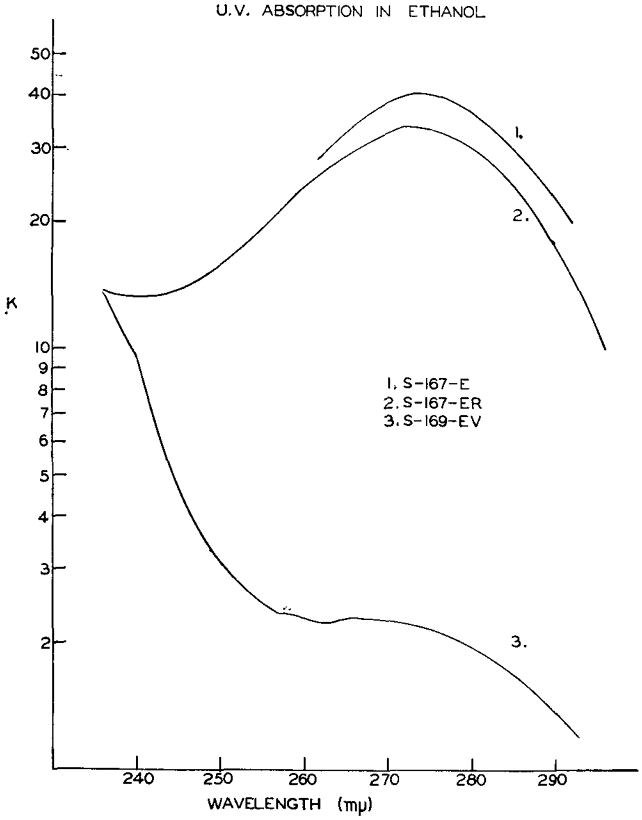


FIG. 2.

microhydrogenations. The leveling bulb contained mercury, and the double column and flask were surrounded by water jackets. The flask was equipped with an oblique-bore stopcock, which was locked in place by a "vacuum adapter." The hydrogen was passed from the tank through Fieser's solution (6), a drying tower, a solvent saturator containing absolute ethanol, an open trap, and then into the buret.

The catalyst was palladium supported on barium sulfate. It was prepared according to the method of Schmidt (7) and contained 5% palladium. The weight of catalyst used was usually about 70% of the sample weight. The catalyst was saturated with hydrogen prior to the addition of the sample.

A sample of each of six condensate fractions was dissolved in absolute ethanol and the amount of hydrogen absorption measured. The volume was corrected to standard conditions, taking into account the atmospheric pressure, temperature, and vapor pressure of the solvent. The hydrogen index (ml. of hydrogen/g. of sample) was calculated. The results are shown in Table I.

A colorimetric method for the determination of α,β-unsaturated aldehydes and ketones has recently been developed by Wearn, Murray, Ramsey, and Chandler (8). This procedure was modified for use as a simple, qualitative test for α,β-unsaturated carbonyls. m-Phenylenediamine dihydrochloride (2.000 g.) was placed in a 50-ml. volumetric flask and made to volume with distilled water, giving a slightly pink solution of concentration 0.040 g./ml. Samples of the various fractions were tested before and after hydrogenation. Samples were drawn up in capillaries of 0.8-mm. bore to a length of 0.4 to 0.5 cm. Each sample was dissolved in 1 ml. of ethanol in a small test-tube and then 1 ml. of the stock reagent solution added. Each tube was shaken and then judged "positive" or "negative" within the next minute. A slight yellow color was considered negative while a dark, golden brown to red was considered positive. A blank was run at the same time. All fractions showed positive tests before hydrogenation while all were negative or showed only traces of α,β-unsaturation after hydrogenation.

Flavor tests were made on the various fractions before and after hydrogenation, using the evaluation procedure developed at the Northern Regional Research Laboratory of the Department of Agriculture (9). All fractions (before hydrogenation) were compared with mineral oil. All were found to have disagreeable flavors, the volatile fractions being more intense than the others. This was also the case when volatile fractions were compared with their respective residue fractions. The volatile fractions were then compared before and after hydrogenation. In the case of both the ether-volatile and the water volatile the hydrogenated samples were found to contain much less flavor, indicating that hydrogenation served to destroy a large portion of the flavor compounds.

Samples of each fraction after hydrogenation were dissolved in ethanol and absorption spectra in the ultraviolet region determined. The maxima which were present in all fractions except S-169-EV in the region of 272 mμ. (Figures 2 and 3) were now completely absent, and general absorption in the entire region studied was decreased tremendously. The spectrum of S-167-ER is given as an example, S-167-ERH being the same fraction after hydrogenation (Figure 4).

6. *Fractionation of the Ether-Soluble-Residue (S-167-ER).* In order to determine what types of compounds might be found in the fractions possessing flavors, the ether-soluble-residue (S-167-ER) was employed in a preliminary exploration before investigating the potent ether-soluble-volatile fraction (S-169-EV). Particularly advantageous in this was the fact that quite a large quantity of S-167-ER was available. (See Figure 1 for a summary of these separations.)

A quantity of 5.0008 g. of S-167-ER was dissolved in 50 ml. of ethyl ether and washed three times (a total of 200 ml.) with 5% potassium carbonate solution. The water phase was separated, washed with fresh ether, and the washings added to the ether solution. The water solution was acidified with 5% hydrochloric acid and extracted with ether until no color remained (six times). This extract was dried over sodium sulfate and the ether removed, leaving the acid fraction, S-225-A, weight 0.8911 g. A test for α,β-unsaturated carbonyls and a Tollens' test were both negative.

The original ether solution was washed with 5% hydrochloric acid and then water; the washings be-

TABLE I

Hydrogenation Results

Sample	Weight (g.)	% Catalyst	Ml. of Hydrogen	Hydrogen Index (ml./g.)
S-167-E	0.0625	65.0	6.88	110.1
S-169-EV	0.0431	69.4	8.32	193.0
S-167-ER	0.0923	76.3	5.22	56.6
S-168-W	0.0442	68.8	2.60	58.8
S-170-WV	0.0389	76.6	2.52	64.8
S-168-WR	0.0403	100.0	1.30	32.3

ing returned to the water solution mentioned above. After drying over sodium sulfate the ether was removed, leaving the neutral fraction, S-225-N, weight 3.2701 g. A test for α,β-unsaturated carbonyls and a Tollens' test were both positive.

Flavor tests on these two fractions showed the typical reverted flavor to be present in S-225-N, the neutral fraction, while S-225-A, the acid fraction, possessed a "burnt" flavor which was not typical of reversion.

In a study of the ultraviolet absorption spectra S-225-N exhibited a maximum at 272-4 mμ. while S-225-A had a maximum at 258 mμ. The specific extinction values were 3.34 and 8.22, respectively. An overall loss in absorption at 272 mμ. of 75% was noted in comparison with that of the original material, S-167-ER.

Fraction S-225-N (2.1894 g.) was oxidized with silver oxide to convert the aldehydes to acids. Silver nitrate (65 g.) was dissolved in water and silver oxide precipitated by the addition of 15.2 g. of sodium hydroxide. The freshly precipitated oxide was washed with water until neutral. The fraction was dissolved in a small amount of methanol and added to the oxide suspension. The oxidation was begun by adding 0.1 *N* sodium hydroxide and was followed by titration with this solution in the presence of phenolphthalein. The base was added slowly with much stirring until the solution remained basic. The reaction was judged as complete when a volume of 45 ml. of base had been added. The mixture was then filtered through Supercel and paper and filtrate reduced in volume.

The filtrate was extracted seven times (200 ml.) and the ether and water phases separated. The ether solution was dried over sodium sulfate and the ether removed, leaving the neutral fraction minus the aldehydes, S-225-NR, weight 0.3676 g.

The water solution was acidified with 5% hydrochloric acid and extracted four times (200 ml.) with ether. The water phase now contained the water-soluble acids derived from the aldehydes; the ether phase contained the major acids derived from the aldehydes. The ether phase was dried and the ether removed, leaving the acid fraction, S-225-AA, weight 0.6282 g. The water phase was extracted in a liquid-liquid extractor with ether for 48 hours and the ether removed, leaving the acid fraction, S-225-WS.

TABLE II

Comparison of 2,4-Dinitrophenylhydrazones

Compound	M. P. (°C.)	Mixed M. P. (°C.)	Color	λ of Max. U.V. Absorption (mμ.)	Analyses	
					C	H
S-232-H	71.4-72.4	70	Orange	362-3	53.55	6.12
Di-n-Propyl Ketone-DNPH	71.0-71.4		Orange	361-2	53.21 53.06*	6.16 6.18*

* Indicates theoretical values.

TABLE III

Comparison of 2,4-Dinitrophenylhydrazones

Compound	M. P. (°C.)	Mixed M. P. (°C.)	Color	λ of Max. U.V. Absorption (mμ.)	Analyses		
					C	H	N
S-234-E	114.6-115.4	128.5-129	R. O.	377	53.89	4.86	20.17
2-Heptenal-DNPH	131.5-132		R. O.	377	53.17 53.41*	5.23 5.52*	19.03 19.17*

* Indicates theoretical values.

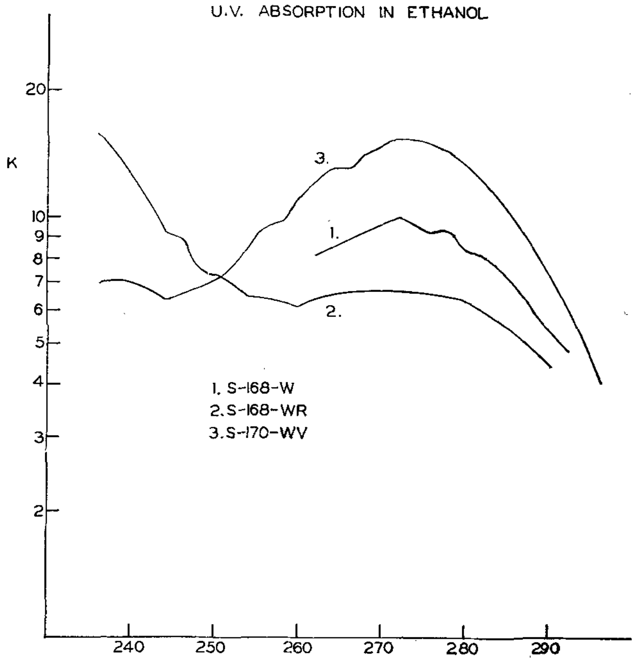


FIG. 3.

All three of these fractions gave negative results when tested for α,β-unsaturated carbonyls and with Tollens' reagent. The only fraction possessing a flavor was S-225-NR, but this was not a reverted flavor.

7. *Fractionation of the Ether-Soluble-Volatile Fraction (S-169-EV).* Since the studies on the ether-soluble-residue above indicated that the flavor materials were present in the neutral fraction, the same type of separation was made on the volatile material (S-169-EV). However, with the anticipation of difficulty in the preparation of acid derivatives, it was decided that the neutral fraction should not be oxidized to the acids. Instead the neutral fraction was converted into carbonyl derivatives (section 8).

A quantity of 2.0140 g. of S-169-EV was dissolved in 50 ml. of ethyl ether and washed three times (125 ml.) with 5% potassium carbonate solution. The water and ether phases were separated and treated in the same manner as in the case of S-167-ER above. The acid fraction, S-230-A, weighed 0.6909 g.; the neutral fraction, S-230-N, weighed 0.6327 g.

The test for α,β-unsaturated carbonyls was positive for S-230-N, but negative for S-230-A. A flavor test paralleled these results. The reverted flavor was found in S-230-N, but not in S-230-A.

An examination of the ultraviolet absorption spectrum of the neutral fraction (S-230-N) in ethanol showed the presence of a wide maximum in the region of 225-230 mμ.

8. *Dinitrophenylhydrazone Reaction on the Neutral Fraction (S-230-N).* Fraction S-230-N (630 mg.) was dissolved in 75 ml. of ethanol and 1,500 mg. of 2,4-dinitrophenylhydrazine added. After heating the mixture to boiling, 3 ml. of concentrated hydrochloric acid were added and the mixture refluxed for 15 minutes. The reaction product was a dark oil mixed with an orange precipitate and excess reagent. The solvent was removed *in vacuo* under nitrogen. The

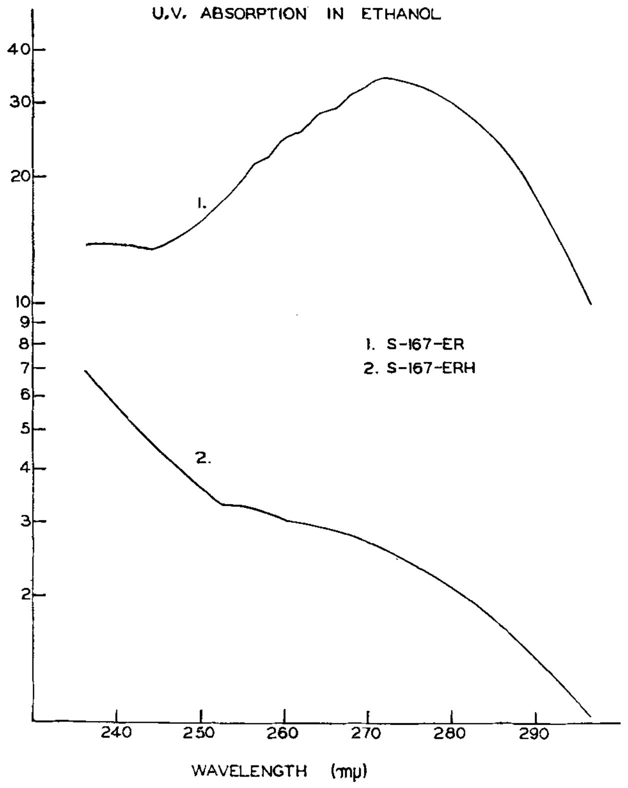


Fig. 4

oily residue was then placed in a small liquid-liquid extractor and extracted with several solvents in succession, and each solution worked up individually.

The reaction residue was first extracted with heptane until no further color change was noticed (two hours). The yellow solution obtained was chromatographed on a column of talc and Super-cel (1:2), using heptane as the developing agent. A wide yellow band passed down the column and was eluted with the heptane. After removal of the solvent, the eluted material was crystallized from ethanol. A small amount of orange crystals was obtained, m.p. 58°C. After several recrystallizations from ethanol and another chromatographic purification, the product melted at 71.4-72.4°C. (S-232-H, Table II), weight 6.6 mg. Analysis of this material showed: C, 53.55; H, 6.12%. A calculation of the empirical formula gave $C_{13}H_{18}N_4O_4$ for the derivative and $C_7H_{14}O$ for the original compound. An examination of the ultraviolet absorption spectrum of S-232-H in ethanol revealed a maximum at 362-363 mμ., which is typical of simple aliphatic aldehyde or ketone derivatives. The low melting point suggested a ketone rather than an aldehyde.

Several samples of commercially available ketones were obtained and dinitrophenylhydrazones prepared. Di-n-propyl ketone-2,4-dinitrophenylhydrazone was found to melt at 71.0-71.4°C. and possessed an absorption maximum at 361-362 mμ. An admixture of this derivative with S-232-H showed no significant depression of the melting point. These comparisons are summarized in Table II. The ultraviolet absorption curves are given in Figure 5.

A sample of the authentic di-n-propyl ketone was dissolved in ethanol and its ultraviolet absorption studied. A sharp maximum at 280 mμ. was found. In a flavor test of the ketone in mineral oil it was described as "fruity" or "painty" and not particularly like reverted soybean oil.

The dinitrophenylhydrazone reaction residue was next extracted in the same apparatus with ethyl ether for seven hours, after which time no further color change was noted. The ether was removed and the remaining oily material refluxed for two hours with petroleum solvent (b.p. 80-90°C.). The yellow solution obtained in this way was chromatographed on a column of talc and Super-cel (1:2) using the same petroleum solvent. A large yellow band was formed and was eluted. After removal of the solvent, the product was allowed to crystallize from ethanol. Red-orange crystals were obtained, m.p. 106-110°C. After rechromatographing and several hot filtrations, the material was recrystallized from ethanol, m.p. 114.6-115.4°C. (S-234-E, Table III), weight 19 mg. Analysis of this derivative showed: C, 53.89; H, 4.86; N, 20.17%. A calculation of the empirical formula gave $C_{13}H_{14}N_4O_4$ for the derivative and $C_7H_{10}O$ for the original compound. The ultraviolet absorption spectrum of a sample of S-234-E in ethanol exhibited a maximum at 377 mμ., characteristic of α,β-unsaturation.

All of the above data indicated that S-234-E and Compound D, which had been isolated previously (1), were identical. Since Compound D had been shown to compare favorably with synthetic 2-heptenal-2,4-dinitrophenylhydrazone, S-234-E was also compared with this derivative. An admixture of the two melted at 128.5-129°C., an intermediate rather than a depressed melting point. Infrared absorption patterns of the two compounds showed a close similarity. Table III summarizes the comparison of data while the ultraviolet and infrared curves are given in Figures 6 and 7 respectively.

Bromination of S-234-E and the synthetic compound was attempted in order to obtain bromo derivatives for further comparisons. This was unsuccessful due to instability of the addition compounds, which evolved

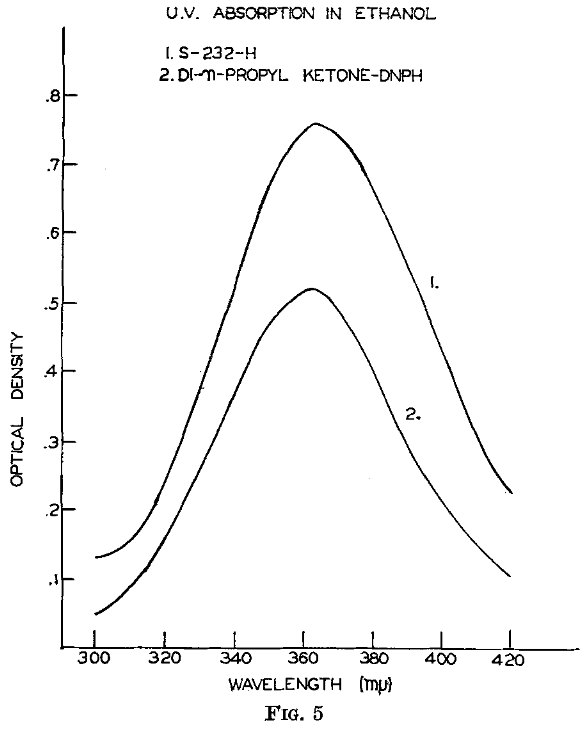


Fig. 5

bromine upon attempted recrystallization. Fractional chromatography of S-234-E failed to produce any fractions with melting points higher than that already reported.

The dinitrophenylhydrazone reaction residue was finally extracted for eight hours with ethanol, almost all of the mass being soluble in it. The extracted material as well as the residue appeared to consist entirely of excess reagent. Polymerization occurred to a great extent, making further work on this material impossible.

9. *Compound S-185-D.* Attempts to purify the dicarbonyl derivatives isolated previously (1) were, for a long time, unsuccessful because of the small quantity of material available and also its very sparing solubility in ethanol and non-polar solvents. This prevented purification by chromatography. A method was finally evolved however which did serve to refine the derivative to some extent (10). The hydrazone was suspended in a solution of 5 ml. of ethanol and 1 ml. of acetone (for the removal of any excess reagent) and 2 drops of concentrated hydrochloric acid added. The mixture was refluxed for 5 minutes. Then 3 ml. of ethanol were added and the mixture filtered hot. The precipitate was washed twice with 2-ml. portions of hot ethanol, then dissolved in 1 ml. of hot nitrobenzene, and allowed to cool slowly. After centrifuging at 1,200 r.p.m., the nitrobenzene was removed by use of a pipet and the precipitate washed six times with 1-ml. portions of ethanol, centrifuging each time. The product was approximately 5 mg. of a microcrystalline, dark red hydrazone, melting at 285-288° C. (S-185-D), Table IV). Analysis: C, 43.70; H, 3.26; N, 24.10%.

When a few crystals of this derivative were treated with alcoholic potassium hydroxide, a blue color was produced, indicating the compound to be a dicarbonyl (11). The derivatives of methyl glyoxal and diacetyl upon similar treatment gave blue and red colors, respectively, while those of single carbonyl compounds, such as crotonaldehyde or heptaldehyde, gave only brown colors.

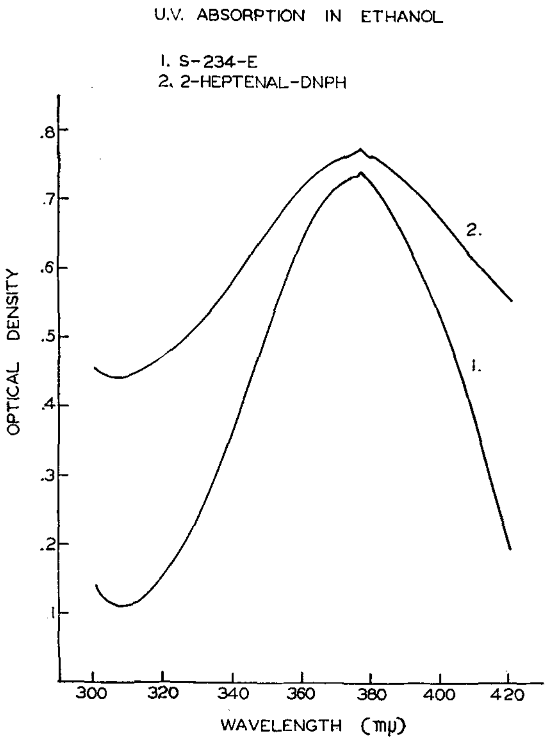


FIG. 6

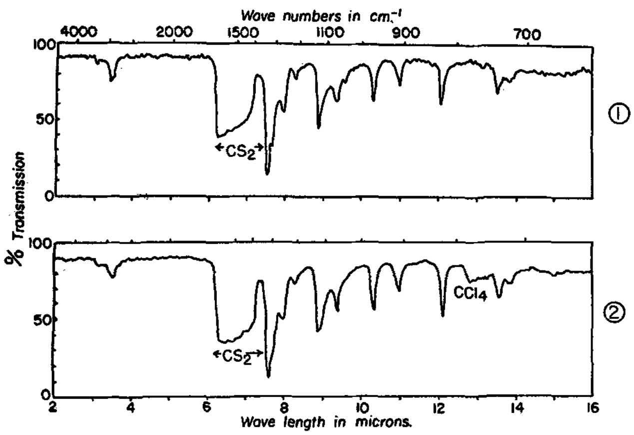


FIG. 7. Infrared absorption curves.
1. S-234-E. 2. 2-Heptenal DNPH.

A calculation of the empirical formula based on the elemental analysis and assuming a dicarbonyl structure resulted in $C_{16}H_{15}N_8O_8$ for the derivative and $C_4H_7O_2$ for the free compound.

An examination of the ultraviolet absorption spectrum of a sample of S-185-D in dioxane revealed a series of maxima in the region of 400-450 mμ. and a major peak at 447-9 mμ. The presence of these absorption maxima at wavelengths higher than those exhibited in the case of saturated dicarbonyl derivatives (10) as well as the color of the derivative suggested the presence of a double bond in conjugation with the carbonyls.

All of the above evidence indicated that S-185-D was a four-carbon, unsaturated dicarbonyl derivative. The simplest possibility was maleic dialdehyde ($C_4H_4O_2$). An authentic sample of maleic dialdehyde-2,4-dinitrophenylhydrazone was obtained (12), and a comparison with S-185-D was made (Table IV). The ultraviolet and infrared curves are shown in Figures 8 and 9 respectively.

Discussion

The validity of the reversion-deodorization technique has been discussed elsewhere (1) and so need not be mentioned here.

1. *Vacuum Distillation.* Vacuum distillation at room temperature was found to be a convenient means of further concentrating the flavor materials. The ether-soluble fraction contained a considerable amount of sterols and tocopherols (4), which were volatile under the conditions of the deodorization. However without the necessary steam and heat these materials were no longer distillable and so did not appear in the volatile fraction. Thus by this simple distillation procedure the smaller, more highly volatile molecules were easily removed from the larger ones. That these small molecules were the ones possessing the flavor was readily shown by the greater intensity of the reverted flavor which the volatile fraction always possessed when compared with the non-volatile residue.

2. *Potassium Carbonate Solution.* Considerable use was made of a separation by 5% potassium carbonate solution. In this way acidic materials were separated from neutral materials. The validity of this procedure as a means of concentrating the flavor compounds was quite evident. The neutral fraction possessed the typi-

TABLE IV

Compound	M. P. (°C.)	Mixed M. P. (°C.)	Color	Color with KOH	λ of Max. Absorption (mμ.)	Analyses		
						C	H	N
S-185-D	285-288	291	Dark Red	Blue	447-9	43.70	3.26	24.10
Maleic Dialdehyde-DNPH	291		Dark Red	Blue	452	42.96 43.25*	2.84 2.72*	25.27 25.21*

* Indicates theoretical values.

cal reverted flavor as well as the α,β-unsaturated carbonyls while the acidic fraction did not. Although losses were found to occur not only in material but also in ultraviolet absorption as a result of this technique, it was felt that these decreases were justified by the separation which was achieved.

3. *Flavors.* It was found that the water-soluble fractions as well as the ether-soluble fractions possessed disagreeable flavors. These flavors were not the same. The typical reversion flavors were present in the ether-soluble fractions while the water-soluble fractions had flavors described as "sharp" or "burnt," bearing little resemblance to the reversion flavor.

When a flavor test was made on an authentic sample of 2-heptenal (1), it was found to bear some resemblance to the flavor of the reverted oil but was difficult to judge because of its intensity. A similar test on di-n-propyl ketone resulted in a flavor described as "fruity" or "painty," not particularly like reverted soybean oil. (Maleic dialdehyde was not available in a free state and so could not be tested.) These results indicated that no one of these compounds is the entire answer to the reversion flavor. It is more likely that the reverted flavor is a composite of these flavors supplemented by flavors of even lesser components in a specific proportion to give the overall result. In this connection it may be noted that the 2-heptenal, which bears more resemblance to the reverted oil than does the di-n-propyl ketone, was found in greater concentration as judged by the yields of the derivatives.

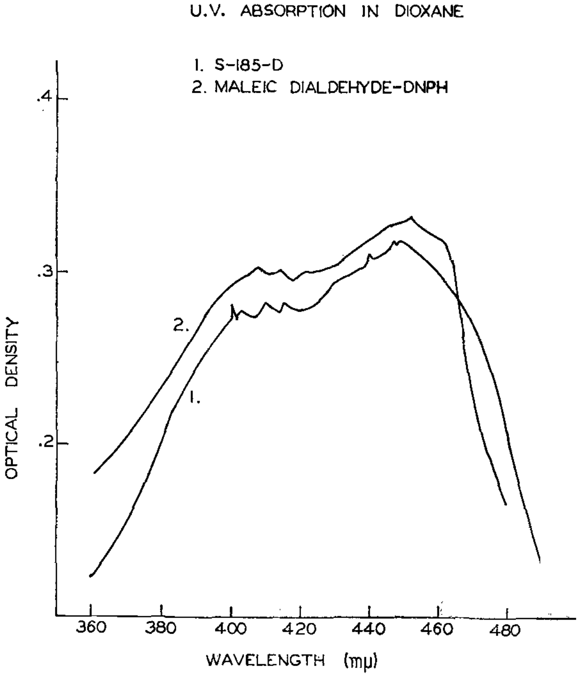


Fig. 8

4. *α,β-Unsaturation.* The test for α,β-unsaturated aldehydes and ketones using m-phenylenediamine was quite helpful. If a fraction possessed a reverted flavor, it always gave a positive test for α,β-unsaturated carbonyls. However the converse was not always true. Thus it was seen in the case of the water-soluble fractions that, although positive α,β-unsaturation tests were obtained, the flavors were not typical of reversion. The test did serve to indicate the type of compounds present in the various fractions, which was well correlated with the fact that α,β-unsaturated materials were later found.

5. *Ultraviolet Absorption Spectra.* It is difficult to state the cause of the absorption at 272 mμ. in view of the fact that neither the authentic 2-heptenal nor the di-n-propyl ketone absorbed in that region. 2-Heptenal had no specific absorption whatever between 220-320 mμ. (1). The di-n-propyl ketone showed a maximum at 280 mμ., but due to its extremely low concentration there is only a slight possibility that the absorption of this compound could enhance the maximum at 272 mμ. The maleic dialdehyde (which was not available in the free state for absorption studies), although possessing a structure which might possibly absorb in that region (13), was also present in too small a concentration to be significant.

Further credence to this non-correlation of absorption at 272 mμ. with the flavor compounds was given in the observation that the absorption maximum decreased as the concentration of flavor components was increased. Thus, the ether-soluble-volatile fraction showed a much lower absorption while containing a greater concentration of flavor materials than the ether-soluble fraction from which it was derived.

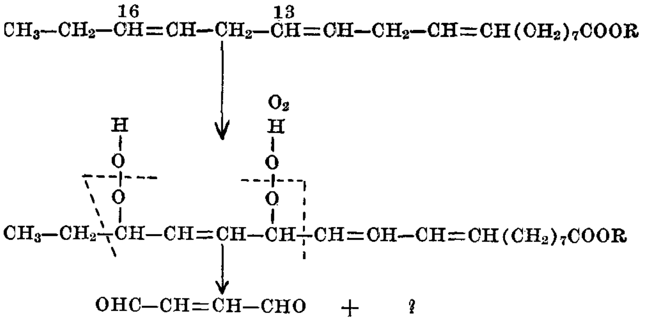
A number of investigators (13-17) have reported the development of an absorption band in the region of 275 mμ. in autoxidizing fat systems. Bolland (16) and Bergström (17) have attributed the observed absorption to a conjugated diene ketone while Evans and Gillam (18) have favored a $\Delta^{2:4}$-dienal. In recent work Swift *et al.* (19) succeeded in isolating $\Delta^{2:4}$-decadienal from autoxidized cottonseed oil and observed that it absorbed at 273-274 mμ. This type of compound may also be the cause of the absorption in the hydrogenated soybean oil condensates, but this seems somewhat unlikely due to the fact that it was not isolated from the volatile fraction in previous work (1) even though that fraction possessed some absorption at 272 mμ.

Perhaps a more plausible answer to the problem lies in the earlier work of Golumbic, Martin, and Daubert (4) in which they succeeded in separating the material exhibiting the absorption maximum by means of chromatography. This material was identified as tocoquinone by conversion to tocopherol. That tocoquinone may be present in reverted soybean oil is quite logical since its formation from tocopherol in oxidizing fats is well-known.

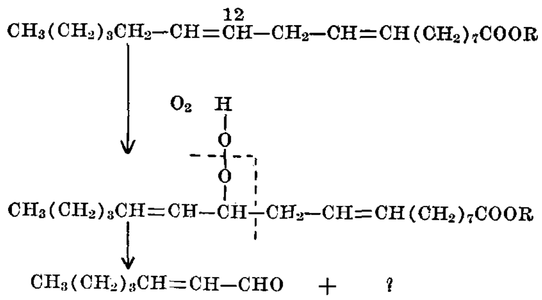
6. *Hydrogenation.* Hydrogenation of the various fractions clearly showed the contribution of α,β-unsaturated carbonyls to the reverted flavor. When these structures were destroyed, the flavor was destroyed. The hydrogen index (Table I) was a good indication of the relative concentrations of flavor materials in the fractions. The highest index was found in the ether-soluble-volatile fraction (S-169-EV) and, as already determined, this fraction contained the greatest concentration of flavor. It may also be noted that hydrogenation effectively destroyed the material causing the absorption maximum at 272 mμ.

7. *The Compounds Isolated.* All of the physical and chemical evidence accumulated indicated the presence of di-n-propyl ketone and maleic dialdehyde in the reverted oil. This was also true of 2-heptenal with the exception of the melting point of the derivative, which could not be raised by any means attempted. An explanation of this anomaly can be made. It is entirely possible that the impurity present in the isolated material is so similar to the heptenal derivative that it is not removed by recrystallization or fractional chromatography and is in such small concentration as to be undetected in the ultraviolet or infrared spectra. Such melting point discrepancies are often found in the literature dealing with the isolation and identification of natural products (20, 21, 22).

As to the origin of these compounds, all one may do is speculate, using the best theories at his disposal. A recent and most promising theory of autoxidation of fats is the hydroperoxide hypothesis of Farmer, Koch, and Sutton (23). If this theory is employed in the case of linolenic acid, one of the dihydroperoxides possibly found is the 13,16-compound. Decomposition along the lines shown below could result in the formation of maleic dialdehyde.



In the same manner, if we take linoleic acid and assume hydroperoxide formation at position 12, 2-heptenal is possible as a decomposition product.



Formation of the di-n-propyl ketone cannot be accounted for by a mechanism of this type.

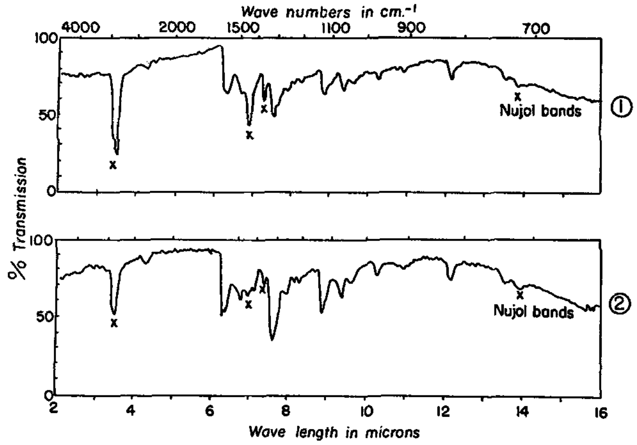


FIG. 9. Infrared absorption curves.
1. S-185-D. 2. Maleic dialdehyde DNPH.

Summary

A reinvestigation of the deodorization condensates obtained from reverted, hydrogenated soybean oil has been made. The flavor materials were further concentrated by vacuum distillation at room temperature and by separation into neutral and acid fractions.

Qualitative tests indicated that the flavor components were mainly α,β-unsaturated carbonyls.

Hydrogenation of the fractions possessing flavors destroyed the α,β-unsaturated carbonyls as well as the flavors. Oxidation of the carbonyls to the corresponding acids gave similar results.

Three compounds were isolated as the 2,4-dinitrophenylhydrazones. They were identified as maleic dialdehyde, di-n-propyl ketone, and 2-heptenal by the use of physical as well as chemical data.

A possible mechanism for the formation of two of these compounds from fatty acid precursors has been indicated.

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