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

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Introduction

IN a previous paper (1) of this series there was reported the isolation of 2-heptenal and a dicarbonyl compound * which contributed to the reverted flavor in soybean oil. The purpose of the present communication is to report further progress on the isolation and identification of other compounds from reverted soybean oil.[3]

For our study of the factor involved in the flavor reversion of soybean oil, it has been our purpose to isolate where possible a) the compound or compounds from condensate fractions whose flavor most closely approaches the flavor of reverted oil and b) the compound or compounds from condensate fractions whose flavor is not directly associated with the reversion. The purpose of investigating b) above is twofold. First, as was indicated previously (1), a true assessment to reversion of any one component may be made only when all of the "reversion compounds" have been isolated and identified. There is the possibility that condensate fractions, although not possessing the typical reverted flavor of the oil, may contain components which when combined with the more evident flavor constituents may enhance their undesirable flavor characteristics. Secondly, with a knowledge of all the breakdown products it may be possible to verify or refute present day theories of the cause of reversion or postulate perhaps a new hypothesis and thereby contribute to a solution of the soybean oil flavor reversion problem.

Experimental

Reversion and Deodorization. The off-flavors present in reverted soybean oil were removed by steam deodorization for 45 minutes at 200°C. and 1-2 mm. pressure, using the apparatus designed by Bailey and Feuge (2), as previously described (1). Two hundred and seventeen kilograms of unhardened oil were used in batches of 14 kilograms. Each batch was subjected to 15 successive operations of heat reversion and deodorization. The total condensate, as collected, was extracted with aldehyde-free ethyl ether (Figure 1). Thus the condensate was divided into an ether phase and a water phase. The water layer (800 ml.) and the ethyl ether layer (800 ml.) were separated by a siphon arrangement and the ether layer successively washed with 500 ml. of water. The ether-soluble fraction, E, was then dried over anhydrous Na_2SO_4 and Drierite. The solution was then filtered and the ether removed by aspiration in an atmosphere of purified nitrogen. This ether-soluble fraction, E, weighed 37 g.

Water Soluble Condensate. The removal of the material in the water phase was effected by a) freezing out the water and filtering, b) liquid-liquid extraction using ethyl ether and c) saturating the water with $(NH_4)_2SO_4$ and extracting with ethyl ether. The weight of this water soluble material (W), was 3.1 g.

Vacuum Distillation of the Ether- and Water-Soluble Fractions. Since previously it has been found that the "reversion compounds" were volatile (3), the water- and ether-soluble fractions were subjected to a room temperature, vacuum distillation (1-3 mm.) with volatile components being trapped in a dry ice container. Agitation was effected by a magnetic stirrer. As a result of this treatment, four fractions were obtained a) ether-soluble volatile, EV, b) ether-soluble residue, ER, c) water-soluble volatile, WV, and d) water-soluble residue, WR.

In an attempt to concentrate the carbonyl material, fraction ER was subjected to a carbonate wash using 5% K_2CO_3. There resulted from this treatment a neutral fraction (Figure 1). Fraction WV was not treated in the same manner because of the small amount of material.

Spectrophotometric analyses were made on EV, ER, WV, and WR, also on the original fractions E and W, using a Beckman Model DU quartz Spectrophotometer (Table I).

Fraction ERN (3 g.) was dissolved in 50 ml. of 95% ethyl alcohol, 3.1 g. of 2,4-dinitrophenylhydrazine added, and the mixture was heated to boiling on a water bath and refluxed for five minutes, after the addition of 1 ml. of concentrated hydrochloric acid. A hot filtration was made and a granular brown-red precipitate was collected in a Buchner funnel. This precipitate was extracted successively with a) heptane (80-90°C.), b) ethyl ether, and c) 95% ethyl alcohol.

The heptane extraction yielded too small an amount of material to be handled adequately.

The alcohol extract was found to contain excess reagent.

The volume of the ether fraction was volatilized completely under reduced pressure and a polymerized-like material remained. This material was refluxed with heptane and the solution decreased in

TABLE I

Spectrophotometric Analysis of Fractions and Compounds Separated From Reverted Soybean Oil

Fraction	λ of Maximum Absorption (mμ.)	Specific Extinction Coefficient (k)	Compound	λ of Maximum Absorption (mμ.)	Specific Extinction Coefficient (k)
E	273	67.6	C	390	110.4
ER	274	66.9	D	294	171.0
ERN	275	8.5	F	390	110.0
EV	275	13.9	G	357	65.8
W	273	8.1	Acetaldehyde-2,4-dinitrophenyl hydrazone	357	66.5
WR	266	7.8			
WV	266	1.3	$\Delta^{2:4}$-decadienal,	293-294	162.7
			semicarbazone(6)	294-295	198.5

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[1] Contribution No. 745 from the Department of Chemistry, University of Pittsburgh.

[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.

* The dicarbonyl compound has since been identified as maleic dialdehyde, Martin and Daubert, Unpublished Results, University of Pittsburgh 13, Pa.

[3] The refined soybean oil used in this investigation was generously supplied by the Humko Company, Memphis, Tenn.

TABLE II

Identification of Carbonyls

Fraction	Derivative	Compound	M.P. °C.	M.P. of Authentic Specimen °C.	Mixed M.P. °C.	Aldehyde Component Identified	Composition						Empirical Formula
							Found			Calculated			
							C	H	N	C	H	N	
ERN	2,4-DNPH[1]	C	140.8-141.2	....	....	$\Delta^{2:4}$-decadienal	57.95	5.70	16.81	58.00[3]	6.04	16.91	$C_{10}H_{16}O$
	Semicarbazone	D	180-181	....	....	$\Delta^{2:4}$-decadienal	62.06	9.39	19.67	63.15[4]	9.09	20.09	$C_{10}H_{16-18}O$[5]
	Hydrogenation of semi-carbazone, D, and converted to 2,4-DNPH	Hydrogenated C	100.8-101.2	103.3[2]	102	Decanal	57.39	7.20	16.00	57.14	7.10	16.61	$C_{10}H_{20}O$
	Semicarbazone, D, converted to 2,4-DNPH	F	137	....	....	$\Delta^{2:4}$-decadienal	57.50[3]	5.97	17.14	58.00	6.04	16.90	$C_{10}H_{16}O$
WV	2,4-DNPH	G	164	164.5[5]	164.2	Acetaldehyde	42.95	3.30	25.46	42.85	3.59	25.04	C_2H_4O

[1] 2,4-dinitrophenylhydrazone. [2] Melting points of 2,4-dinitrophenylhydrazone of decanal and acetaldehyde reported to be 104°C. and 164-164.5°C. respectively (7, 8). [3] Calculated for 2,4-dinitrophenylhydrazone of $\Delta^{2:4}$-decadienal. [4] Calculated for semicarbazone of $\Delta^{2:4}$-decadienal. [5] The formula $C_{10}H_{17}O$ obtained by calculation was found to be impossible.

volume under reduced pressure. A red-orange precipitate was collected.

This precipitate, Compound C (Table II), was dissolved in heptane and chromatographed on a talc-Supercel (1:2) column (30 x 1.5 cm.). The developer and eluent was a mixed solvent containing heptane and benzene (9:1). Two bands were noted: a red-brown band I (0.5 cm.) at the top of the column and an orange-yellow band II (5 cm.) about 3 cm. down the column. Band II was Compound C, m.p. 131-135°C.

After removal of the solvent in vacuo from the filtrate remaining after removal of the precipitate above, a gummy polymerized material was obtained. This material was extracted with hot heptane. On cooling the solution, a red-orange precipitate was collected on filtration. A portion (30 mg.) of this precipitate was dissolved in heptane and chromatographed in the same manner as above. Compound C, m.p. 138°C., was obtained. Finally a constant melting Compound C was obtained by chromatographing again, as above, and after three crystallizations from heptane. M.P. 140.8-141.2°C. Crystalline form, red-orange platelets. Analysis: C, 57.95; H, 5.70; N, 16.81% (Table II).

An absorption maximum at 390 mμ. was observed for the crystalline derivative C. (Table I). In an effort to further elucidate the structure of Compound C, it was thought that more information could be obtained by the preparation of the semicarbazone.

Preparation of the Semicarbazone. Semicarbazide hydrochloride (1.2 g.) and 1.8 g. of hydrated sodium acetate were ground together until a paste formed and 10 ml. of methanol were added. This solution was filtered into a flask containing 2.5 g. of fraction ERN. Methanol (15 ml.) was added and the solution allowed to stand at room temperature. A white powder-like precipitate, Compound D, came out of solution and was filtered off (40 mg.), m.p. 179-180°C.

A second batch of fraction ERN (2.0 g.) was treated in a similar manner. Again a white powder-like material was obtained, m.p. 180°C. The precipitate, Compound D, from both reactions were combined. Much difficulty was encountered in attempting to purify this compound by crystallization from ethyl alcohol or benzene. Finally the material was chromatographed on a talc-Supercel (1:2) column, (20 x 1.5 cm.) using heptane as the solvent throughout the procedure. A semi-crystalline, Compound D, was isolated, m.p. 180-181°C. Analysis: C, 62.06; H, 9.39; N, 19.67% (Table II). The absorption spectrum of Compound D showed a maximum at 294 mμ.

Since oxidation with potassium permanganate, as well as a consideration of the empirical formula of Compounds C and D, indicated unsaturation, hydrogenation of Compound D was selected as the next procedure.

Hydrogenation of Compound D. Twenty-five milligrams of Compound D were dissolved in 95% ethyl alcohol and 20 mg. of palladium-on-barium sulfate were added. The hydrogenation was carried out at room temperature and atmospheric pressure, with agitation effected by a magnetic stirrer.

After hydrogenation, the solution was then treated with 10 drops of concentrated hydrochloric acid and 25 mg. of 2,4-dinitrophenylhydrazine. After refluxing on a hot water bath for five minutes, the volume of solution was reduced in vacuo and a yellow precipitate formed, which was then filtered. The precipitate was dissolved in heptane and chromatographed on a talc-Supercel column (1:2), as previously described in the handling of Compound C. The yellow derivative, hydrogenated Compound D, was obtained from the eluate. M.P. 96°C. Subsequent crystallization (3x) from 95% ethyl alcohol raised the melting point to 100.2-100.4°C. A mixed melting point of hydrogenated Compound D and an authentic sample of the 2,4-dinitrophenylhydrazone of decanal (m.p. 103.3°C.) was 100.8-101.2°C.(Table II). The absorption maximum at 359 mμ., observed for hydrogenated Compound D, was identical with the maximum observed for n-decanal-2,4-dinitrophenylhydrazone.

In order to determine that Compounds D and C were derivatives of the same carbonyl, Compound D (10 mg.) was hydrolyzed with 6 drops of concentrated hydrochloric acid, and reacted with 8 mg. of 2,4-dinitrophenylhydrazine in the usual manner. A red-orange crystalline, Compound F, m.p. 137°C. was isolated. The mixed melting point of Compound F and C was 139.5°C. Analysis: C, 58.00; H, 6.04; N, 16.91%. An absorption analysis of Compound F showed a maximum at 390 mμ.

Fraction WV. Fraction WV (0.5 g.) was dissolved in 35 ml. of 95% ethyl alcohol, 0.4 g. of 2,4-dinitro-

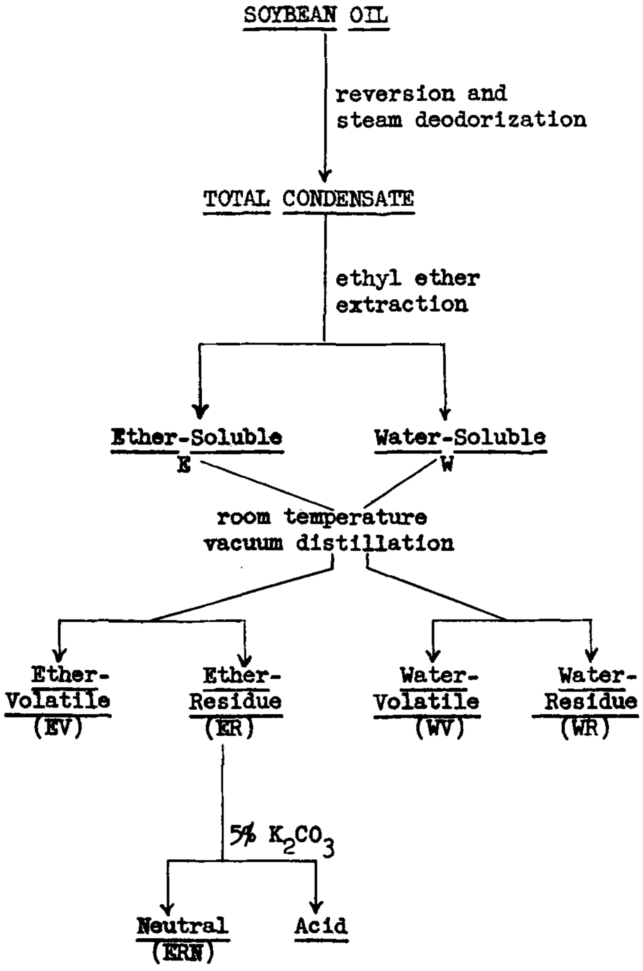

FIG. 1.

phenylhydrazine added, and the mixture heated to boiling and refluxed for five minutes, after addition of 1 ml. of concentrated hydrochloric acid. The brown-yellow precipitate which deposited from solution was collected on a Buchner funnel on filtration of the solution. The precipitate was dissolved in heptane and chromatographed. The chromatography was carried out on a column (45 x 1.2 cm.), using an adsorbent of silicic acid and Supercel in a ratio of 2:1. The developing solvent was a mixed solvent containing heptane and benzene in a ratio of 9:1. After development two bands appeared: a red-brown Band I (0.2 cm.) at the top and an almost colorless band (2 cm.), followed by an orange-yellow Band II. Band II was eluted with a mixed solvent consisting of heptane and ether in a ratio of 10:1. A yellow compound, G, was isolated and purified to a constant melting point of 164°C., by crystallization from 95% ethyl alcohol. Analysis: C, 42.95; H, 3.30; N, 25.46%. The absorption maximum observed for Compound G at 357 mμ. was the same as that observed for the 2,4-DNPH of acetaldehyde (Table I).

Discussion

The off-flavors present in reverted soybean oil were removed by vacuum distillation under reduced pressure. The steam volatile condensate, thus obtained, consisted of an ether- and water-soluble phase. Since, as mentioned previously, it was found that "reversion compounds" were volatile (3), the above ether- and water-soluble material were subjected to a room temperature, vacuum distillation.

The resulting fractions thus obtained, were then considered for flavor, absorption studies, as well as their chemical composition as far as carbonyl constituents were concerned.

The ether-volatile material (EV) exhibited flavor characteristics closely approaching that of reverted soybean oil whereas other fractions did not possess a flavor typical of soybean oil reversion.

Previously it was shown (1), in the case of the ether-volatile material, that the carbonyl portion possessed the flavor which was similar to reverted soybean oil. Consequently the ether-soluble residue material was treated with 5% K_2CO_3. It was again found that what flavor characteristics the original material possessed were found, for the most part, in the neutral material isolated.

Purification of the 2,4-dinitrophenylhydrazones of the flavor components was best effected by chromatography. Subsequent crystallization from the eluate solution resulted in the formation of well-defined crystals.

Further elucidation of the structure of Compound C, Table II, was effected through the formation of the corresponding semicarbazone, Compound D. The semicarbazones, where conditions are favorable for their utilization, are much more versatile derivatives than the 2,4-dinitrophenylhydrazones. Thus by hydrogenation of Compound D and subsequent hydrolysis and formation of the 2,4-dinitrophenylhydrazine, the skeleton structure of Compound C (a straight chain ten-carbon aldehyde) could be determined (Table II).

The absorption data, as reported in Table I for the various carbonyl derivatives considered, were relied on to a large extent in the elucidation of the structure of Compound C. The absorption maxima at 294 mμ. and 390 mμ. observed for Compounds D and C, respectively, are associated with $\Delta^{2:4}$-dienals (5, 4). The specific extinction coefficient, k, reported (6) for $\Delta^{2:4}$-decadienal-semicarbazone was 162.7 at 293-294 mμ. and 198.5 at 294-295 mμ. for a purer sample. The k value observed by us was 171.0 but, as already noted, some difficulty was encountered in the purification of Compound D.

The physical and chemical data related to Compound C are listed in Table II. Observation of these data, including the comparison of Compound D with the semicarbazone of $\Delta^{2:4}$-decadienal isolated by Swift *et al.* (6), suggests the structure of Compound C to be the 2,4-dinitrophenylhydrazone of $\Delta^{2:4}$-decadienal.

The physical and chemical data compiled for compound G (Table I, II) are very similar to those observed for acetaldehyde-2,4-dinitrophenylhydrazone. The absorption maxima at 357 mμ. and the k value of 66.8 are comparable to the authentic specimen. As further substantiation of the structure of Compound G, infrared curves[4] of Compound G, and the authentic specimen were compared. The curves were very similar.

As to the contribution of $\Delta^{2:4}$-decadienal and acetaldehyde to flavor reversion, this has not as yet been established. The explanation of the part possibly played by these compounds in flavor reversion in reverted soybean oil has been stated in the Introduction.

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[4] Infrared curves were obtained through the courtesy of the Mellon Institute Spectroscopy Laboratories.

Swift *et al.* (6), working with autoxidized cottonseed oil, isolated $\Delta^{2:4}$-decadienal, Δ^{2}-octenal and n-hexanal. According to Swift's postulation, linoleic acid is the precursor being decomposed through the formation of the isomer hydroperoxides. However, the formation of Δ^{2}-octenal and n-hexanal has not as yet been observed in this laboratory.

Summary

Two compounds possibly contributing to the reverted flavor in soybean oil have been isolated from the condensate obtained from the deodorization of soybean oils.

From physical and chemical data compiled, including absorption and hydrogenation studies, the structure of the 2,4-dinitrophenylhydrazones have been shown to correspond closely with the 2-4-dinitrophenylhydrazones of $\Delta^{2:4}$-decadienal and acetaldehyde.

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Sesame Oil. V. The Stability of Sesame Oil[1]

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ONE of the significant properties of sesame oil which has been most often noted or remarked upon in recent years is its marked stability towards oxidative rancidity, after hydrogenation. Grettie (1) was granted a patent in 1933 covering the addition of small amounts of *undeodorized*, hydrogenated sesame oil to lard and other fats for purposes of stabilizing the latter fats. In 1937 Grettie (2) showed that the oily material separated from the deodorizer distillate from hydrogenated sesame oil was effective as an antioxidant in lard and other fats.

In the course of an investigation of different oils of pharmaceutical importance Fiero (3,4) found that hydrogenated sesame oil was the most stable of the hydrogenated vegetable oils which he examined. This author (5) also found that hydrogenated sesame oil imparted stability to lard when added in relatively large amounts, but the unsaponifiable fraction from this oil did not exhibit any appreciable antioxidant activity. This result was quite unexpected because, as pointed out by Fiero, the composition of sesame oil did not seem to be essentially different from that of other vegetable oils having similar physical constants.

The marked stability of hydrogenated sesame oil (500 to 900 hours, A.O.M.) was also noted in the first two reports of this series (6,7). The cause of the marked stability of hydrogenated sesame oil has not heretofore been known although Olcott and Mattill (8) suggested that the presence of sesamol might be responsible for this stability. In another report (9) of the present series sesamol has been shown to exert a pronounced antioxidant activity in lard and various vegetable oil substrates. A method for the determination of free and bound sesamol has been described in a fourth report (10) in this series.

In the present report the results of an investigation of the stability of sesame oil are presented with special reference to the role of sesamol in this respect.

Experimental

Properties of the crude, alkali-refined, bleached, hydrogenated, and deodorized sesame oils investigated were described in a previous report (7). Stabilities were determined by the active oxygen method at a temperature of 97.7°C. Free and bound sesamol was determined according to a method (10) previously described.

TABLE I

Relation of Stability of Sesame Oils to Their Contents of Free, Bound, and Total Sesamol

Oil	Stability, A.O.M., hours;[a]	Sesamol,%		
		Free	Bound	Total
SO-1				
Crude	33	0.001	0.144	0.145
Alkali-refined	15	0.000	0.143	0.143
Bleached, official earth[b]	32	0..019	0.069	0.088
Bleached, "B.C. clay"[c]	19	0.014	0.117	0.131
Bleached, acid clay[d]	39	0.075	0.002	0.077
Bleached, neutral clay[e]	43	0.093	0.004	0.097
Bleached, carbon[f]	17.5	0.011	0.079	0.090
Deodorized, (B.C. bleached)	11.0	0.001	0.119	0.120
SO-2				
Crude	19	0.003	0.133	0.136
Alkali-refined	9.5	0.001	0.139	0.140
Bleached, official earth[b]	14.5	0.005	0.079	0.084
Bleached, "B.C. clay"[c]	18	0.011	0.117	0.128
Bleached, neutral clay[e]	56	0.089	0.015	0.104
Deodorized, (B.C. bleached)	8.5	0.001	0.099	0.100
SO-3				
Crude	20	0.001	0.135	0.136
Alkali-refined	9.0	0.001	0.134	0.135
Bleached, official earth[b]	17	0.009	0.080	0.089
Bleached, "B.C. clay"[c]	11.0	0.006	0.124	0.130
Deodorized, (B.C. bleached)	10.7	0.001	0.001	0.002
SO-4				
Crude	4.5	0.001	0.161	0.162
Alkali-refined	6.9	0.001	0.164	0.165
Bleached, official earth[b]	17	0.018	0.110	0.128
Bleached, "B.C. clay"[c]	13.8	0.011	0.151	0.162
Deodorized, (B.C. bleached)	9.3	0.001	0.007	0.008

[a] Time required to attain a peroxide value of 100 m.e./kg.
[b] 5.5% AOCS earth.
[c] 2% clay.
[d] 3% acid type clay.
[e] 3% neutral type clay.
[f] 3% activated carbon.

Stabilities of four crude, alkali-refined bleached, and deodorized sesame oils, together with their contents of free, bound, and total sesamol, are shown in Table I. From the data in Table I it may be seen

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[1] Presented at the 23rd Fall Meeting of the American Oil Chemists' Society, Chicago, Ill., October 31 to November 2, 1949.

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