

The Relationship Between the Oxidative Polymers of Soybean Oil and Flavor Reversion¹

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IN recent studies on "reverted" soybean oil (1, 2, 3) short chained aldehydes and ketones have been isolated from autoxidized soybean oil and identified as the odor and flavor components of the reverted oil. However the precursors of these carbonyl compounds have, as yet, not been identified. These precursors must meet the following four conditions: 1. They must be more readily formed in soybean oil than in cottonseed oil; 2. they cannot be readily removed by present refining and hydrogenation practices; 3. before breakdown, they must not possess the odors or flavors characteristic of reverted oil; and 4. their breakdown is not inhibited by antioxidants and can take place under high vacuum or under inert gases.

Previous papers (4, 5) from this laboratory indicated that the more polar oxidative polymers of ethyl linoleate and linolenate seemed to meet these requirements. In the present investigation the more polar oxidative polymers of soybean oil were isolated and their properties studied in order to reveal the relationship between the oxidative polymers and the problem of flavor reversion.

Experimental

Preparation of Oxidative Polymers of Soybean Oil. Fifteen hundred g. of soybean oil was oxidized in an all-glass apparatus by bubbling filtered dry air through it at 60°C. until the refractive index at 30°C. had increased from 1.4722 to 1.4780. A period of 12 days elapsed before the latter refractive index was attained. Fifteen hundred g. of the autoxidized product was separated into 14 fractions (Table I). The first seven fractions were obtained by repeated extractions with pentane-hexane until the solubility reached a constant value of about 0.4 g. per 100 ml. The next seven fractions were obtained by extractions with solvent pairs made up of pentane-hexane with successively increasing amounts of anhydrous diethyl ether. The extractions were carried out in an all-glass apparatus under an atmosphere of nitro-

gen as reported in a previous paper (6). Fractions 12 and 13 were combined and used as the more polar polymers for this investigation.

Autoxidation of Oxidative Polymers of Soybean Oil. Two batches of 50 g. each of the more polar polymers were oxidized with filtered dry air for 7 days at 30°C. In one batch the polymers were dispersed on glass wool and in the other on strips of filter paper. Effluent air, conveying the volatile decomposition products, was then passed through two glass traps connected in series and immersed in acetone cooled with solid carbon dioxide.

Characterization of the Volatile Carbonyl Compounds. The volatile material collected in the cold traps was washed out with redistilled pentane-hexane (B.P. 40°C.). A total volume of 300 ml. of pentane-hexane washings was extracted four times with 150-ml. portions of water. The water extract, fraction A was fractionally distilled with a 20-in. column packed with glass beads. Twenty-five ml. of fraction C, boiling point 99-100°C., and 50 ml. of fraction D, boiling point 100°C., were collected. The residue E, was saturated with sodium chloride and then extracted with diethyl ether. The ether extract was designated fraction F, and the residue fraction G. The original pentane-hexane soluble fraction B was also fractionally distilled with a 20-in. column packed with glass beads. Fraction H, boiling point 35-38°C., fraction I, boiling point 38-39°C., and fraction J, boiling point 39-40°C., were collected. The residue was designated as fraction K.

Each of the fractions was converted into 2,4-dinitrophenylhydrazones by the method of Gordon *et al.* (7). The 2,4-dinitrophenylhydrazones from each fraction were separated by the chromatographic procedure reported in a previous paper (4). Each of the isolated 2,4-dinitrophenylhydrazones was rechromatographed, recrystallized, and then identified by comparing its color, melting point, mixed melting point, maximum absorption, and ultimate analysis with those of authentic samples.

Depolymerization Procedure. Two hundred fifty g. of a polymeric substance obtained by scraping the filter cloth of the presses of a modern large soybean

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TABLE I
Chemical and Physical Properties of the Solvent-Extracted Fractions of Autoxidized Soybean Oil

Fraction	Solvent			Weight (grams)	Refractive index $n_D^{30^\circ C.}$	Molecular weight (cryoscopic)	Viscosity (centipoise at 30°C.)	Peroxide number (millimoles/kg.)
	Ml.	% Pentane-hexane	% Diethyl ether					
1.....	12,000	100	0	968.1	1.4751	361	687.5
2.....	3,000	100	0	92.4	1.4742	246	719.9
3.....	3,000	100	0	39.5	1.4769	448	842.0
4.....	3,000	100	0	21.2	1.4779
5.....	3,000	100	0	18.7	1.4782	978.0
6.....	3,000	100	0	11.3	1.4785	1026
7.....	3,000	100	0	11.0	1.4785	1091
8.....	3,000	90	10	24.1	1.4798	1113
9.....	3,000	80	20	49.3	1.4827	1,510	1279
10.....	3,000	70	30	72.2	1.4849	1135	2,724	1278
11.....	3,000	60	40	57.8	1.4857	7,480	1355
12.....	3,000	50	50	73.8	1.4870	2221	18,300	1267
13.....	3,000	40	60	49.3	1.4889	2832	30,500	1120
14.....	300	0	100	4.5	Jel	Jel	1086

TABLE II
 Identification of the 2,4-dinitrophenylhydrazones

	Color	Melting point, °C.	Mixed melting point, °C.	Maximum absorption, $m\mu$	Ultimate analysis		
					C%	H%	N%
C-I-I.....	Yellow	165	165-167	356	43.02	3.81	24.67
Acetaldehyde.....	Yellow	168		356	42.86	3.60	24.99
C-I-II.....	Orange	145-150	148-151	356	45.79	4.37	23.08
Propionaldehyde.....	Orange	155		356	45.38	4.23	23.52
F-I.....	Red	160-163	161-163	374-375	50.74	4.79	21.01
2-Pentenal.....	Red	162-163		375	50.30	4.57	21.19
K-II.....	Yellow	105	106-107	356	51.83	6.02	19.58
n-Hexanal.....	Yellow	107		356	51.40	5.78	20.01

oil refinery (Figure 2) was extracted six times with pentane-hexane in a Waring blender under nitrogen to remove unpolymerized oil trapped in the gel. The residue was dried under vacuum at room temperature. One hundred and ninety-five g. of sticky powder was obtained.

Depolymerization and ethanolysis of this sticky powder were carried out in ethyl alcohol, which had been adjusted to a normality of 3.5 with anhydrous hydrogen chloride. One hundred and seventy-six g. of the polymers formed in the filter press and 1,760 ml. of 3.5 N. hydrochloric acid in 95% ethyl alcohol were introduced into a round bottom flask and stirred vigorously. The flask was connected to a reflux condenser and heated to 80°C. by means of an oil bath. After two hours the reaction mixture was cooled under tap water and diluted with an equal volume of distilled water. The mixture was extracted three times with an equal volume of redistilled pentane-hexane. The combined extracts were washed with distilled water until free of acid, dried over anhydrous sodium sulfate, decolorized with activated carbon, and freed from solvent under vacuum at room temperature. One hundred and twenty g. of depolymerized product was obtained.

The polymer fractions 12 and 13, which were isolated from soybean oil autoxidized at 60°C., were depolymerized by the same treatment. Fourteen and one-tenth g. of depolymerized product were obtained from 22.4 g. of the more polar polymer fractions.

Analytical Procedures. Molecular weights were determined by the cryoscopic method with benzene as solvent (6). Viscosity was measured with a Brookfield viscometer. The presence of carbonyl compounds was tested by the method of Lappin and Clark (8). Oxygen was determined by the method of Pregl as described by Grant (9).

Results

The chemical and physical properties of the solvent-extracted fractions indicated that it was possible to separate autoxidized soybean oil into fractions of increasing polarity and molecular weight by the solvent-extraction method (Table I). As the concentration of diethyl ether in pentane-hexane was increased from 0 to 100% the refractive index, molecular weight, and viscosity of the various fractions gradually increased. The peroxide number increased to a maximum of 1,355 for fraction 11 and decreased to 1,086 for fraction 14.

The oxidative polymers were insoluble in nonpolar solvents although the presence of a large amount of monomers increased the solubility of the polymers in these solvents. When the dilution of a solution of a

mixture of monomers and polymers in a non-polar solvent was sufficiently large, the influence of the monomers decreased and the polymers separated out. For example, 1 g. of autoxidized soybean oil which contained approximately 1% of polymers was miscible with any amount of pentane-hexane from 0 to 45 ml. However if more than 45 ml. of pentane-hexane was added, the polymers separated out. It is therefore evident why 12 liters of pentane-hexane were required for the first extraction.

Five carbonyl compounds were identified in the volatile decomposition products obtained by reautoxidizing the more polar polymers of soybean oil (Table II). The 2,4-dinitrophenylhydrazones obtained from fraction C were separated into two aldehydes by chromatography. One was identified as acetaldehyde and the other as propionaldehyde. Fraction D gave only 15 mg. of 2,4-dinitrophenylhydrazones. On chromatographing, this fraction separated into one orange, one yellow, and one diffused yellow band. None of these bands contained enough crystalline material for complete characterization. One major component was found in the 2,4-dinitrophenylhydrazones obtained from fraction F. After this component was rechromatographed and recrystallized, it melted at 160 to 163°C. and was identified as the 2,4-dinitrophenylhydrazone of 2-pentenal. Fraction H was separated into two yellow bands and Fraction I into five yellow bands by preliminary chromatography. The small amount of material in each of these bands made complete identification impossible. Fraction J yielded no 2,4-dinitrophenylhydrazones. The 2,4-dinitrophenylhydrazones obtained from Fraction K were resolved into one orange and two yellow bands. The middle yellow band was identified as the 2,4-dinitrophenylhydrazone of n-hexanal. The top orange band was re-

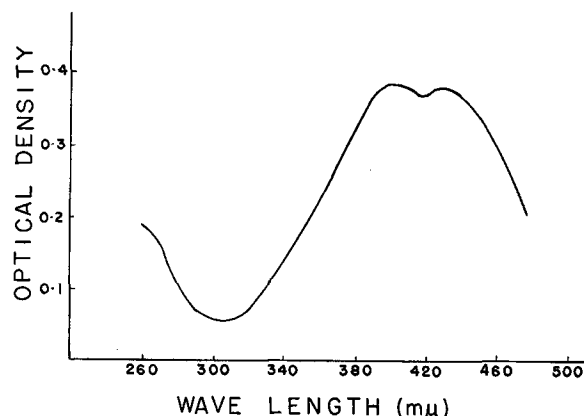


FIG. 1. Absorption spectrum of fraction K-I in anhydrous ethanol.

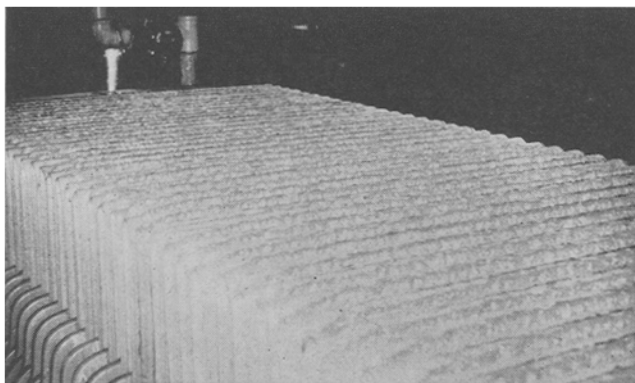


FIG. 2. Top view of one of the filter presses in a large soybean oil refinery showing the accumulation of polymerized oil on the filter cloth.

chromatographed twice and then recrystallized from 95% ethyl alcohol. The absorption spectrum of this 2,4-dinitrophenylhydrazone (Figure 1) was identical with those of dihydrazones of dicarbonyls (10).

The present results indicated that the more polar polymers contained 9.24% more oxygen than the fresh refined soybean oil or 21.04 and 11.80%, respectively. In order to prove that the oxidative polymers could decompose under high vacuum, two tubes, each containing 10 g. of the more polar polymers were evacuated and then released to oxygen-free nitrogen. This operation was repeated three times. Each of the tubes were then evacuated to 200 microns, sealed, and one tube kept at 30°C. and the other at -20°C. After three weeks the tubes were opened; each was washed with 10 ml. of water; and one ml. of the aqueous solution was used for a Lappin Clark test (8) for carbonyl compounds. The solution from both tubes gave positive tests and thus indicated that carbonyl compounds were formed during storage under vacuum. Furthermore the solution obtained from the polymers which had been stored at 30°C. had a higher optical density at 480 $m\mu$ than the one from those stored at -20°C. or 0.603 and 0.188, respectively. Therefore the carbonyl compounds must have formed during storage and were not due to contamination of the viscous polymers by compounds formed previous to sealing.

The oxidative polymers were also shown to decompose under oxygen-free nitrogen. This nitrogen was prepared from high purity tank nitrogen by bubbling the latter through three gas-washing bottles, each containing 800 ml. of a 20% solution of pyrogallol in 15% aqueous sodium hydroxide solution. It was dried by passing it through concentrated sulfuric acid, calcium chloride, and glass wool, and then bubbled through 15 g. of the more polar polymers at 30°C. After the system had been flushed out with purified nitrogen for two hours, the effluent nitrogen was passed through a cold trap immersed in acetone and cooled with solid carbon dioxide. The condensate was removed from the trap during various intervals of time by washing with carbonyl free methyl alcohol. A volume of 3 ml. of methyl alcohol per day was used. A Lappin Clark test for carbonyl compounds was carried out with one ml. of the alcoholic solutions. Positive tests for the solutions obtained from the first to the eighteenth day (Table III)

indicated that the oxidative polymers decomposed slowly under nitrogen with the formation of volatile carbonyl compounds.

The polymers of soybean oil were depolymerized as easily as the polymers of ethyl linoleate (6). The

TABLE III
Degradation of the Oxidative Polymers of Soybean Oil Under Oxygen-Free Nitrogen

Period (day)	Optical density at 480 $m\mu$ for each 24 hours
1	0.510
2	0.640
3-4	0.610
5-6	0.630
7-9	0.342
10-13	0.270
14	0.323
15-18	0.220

sticky powder or pentane-hexane insoluble fraction of the polymeric material which had been deposited on the filter cloth of a soybean oil refinery press as well as the more polar polymer fractions 12 and 13 obtained from autoxidizing soybean oil at 60°C. were depolymerized by treatment with 3.5 N. hydrochloric acid in 95% ethyl alcohol (Table IV). The very

TABLE IV
Comparison of the Properties of the Oxidative Polymers of Soybean Oil Before and After Depolymerization

	Polymers of soybean oils formed at 60°C.		Polymers of soybean oil formed on filter press of a soybean oil refinery	
	Before depolymerization	After depolymerization	Before depolymerization	After depolymerization
Molecular weight (cryoscopic)	2,832	312	*	260
Refractive index $n_D^{30^\circ}$	1.4889	1.4552	Gel	1.4530
Viscosity (centipoise at 30°C.)	30,500	Mobile liquid	Gel	Mobile liquid
Solubility in pentane-hexane	Insoluble	Miscible	Insoluble	Miscible
Peroxide number (millimoles/kg.)	1,120	1.42	*	0

* Insoluble in solvents used for the determinations.

similar manner of depolymerization with the formation of similar depolymerization products indicated that the polymers formed on the filter press and the more polar polymer fractions 12 and 13 might contain the same type of carbon to oxygen polymeric linkages (6).

Discussion

The carbonyl compounds identified in the volatile decomposition products obtained by reautoxidizing the more polar polymers of soybean oil, that is, acetaldehyde, propionaldehyde, n-hexanal, and 2-pentenal have been isolated from reversioned soybean oil by previous workers (1, 2, 3). These aldehydes were also obtained in the volatile decomposition products of the reautoxidized polar polymers of either ethyl linoleate or linolenate (4, 5). It is therefore evident that if oxidative polymers of the glyceryl esters of linoleic or linolenic acid are present in soybean oil, they may serve as precursors of reversion compounds.

Polymers have been reported to form in ethyl linolenate under mild conditions of autoxidation (11).

It is not known however whether polymers are formed in unsaturated triglycerides during the initial stages of autoxidation. Whether polymers are formed during the initial stages or only after prolonged autoxidation seems irrelevant to their function as precursors of reversion components. If formed during the initial stages, they are present in the oil *per se*. If formed only after prolonged autoxidation, the polymers could be preformed and dissolved in fresh soybean oil. For example, the polymeric substance found on the filter press could have been formed from oil previously passed through it. The polymers could then be dissolved by fresh oil as it passes through the contaminated press. However this does not mean that the filter press is the only place where oxidative polymers could be formed and contaminate soybean oil. For example, the deodorizer is another point where oxidative polymers might be formed. If inward leakage is not completely prevented or if the steam is not entirely free of air, the oil in the deodorizer, during the several hours of heating at relatively high temperature with vigorous agitation, may form small amounts of polymers. It is therefore possible that whenever the conditions are suitable, soybean oil could form oxidative polymers. Any odorous compounds formed during polymerization may be subsequently removed by deodorization, but the polymers may remain in the oil and serve as a precursor of reversion compounds.

Golumbic and Daubert (12) pointed out that only a very small amount of oxygen is required to produce reversion. Furthermore Robinson and Black (13) observed flavor deterioration in soybean oil aged under inert gases, and Bickford (14) reported that reversion could occur during storage under high vacuum. All these observations can be explained with the present data. These data showed that the oxidative polymers are 9.24% higher in oxygen content than fresh refined soybean oil and could decompose without further absorption of much more oxygen. Since the oxidative polymers were shown to decompose under high vacuum or nitrogen, the excluding of air could only prevent autoxidation of the unsaturated triglycerides but not the decomposition of the more polar polymers with the resulting formation of volatile carbonyl compounds. It is therefore evident that soybean oil containing small amounts of oxidative polymers could revert when aged under these conditions.

Summary

The polymers formed during the autoxidation of soybean oil at 60°C. were isolated by a solvent-extraction method, using diethyl ether and pentane-hexane as solvents. The more polar polymer fraction was further oxidized by air at 30°C., and the volatile material obtained was fractionated and characterized. It contained the same carbonyl compounds as the volatile material which has previously been obtained from reverted soybean oil, namely acetaldehyde, propionaldehyde, n-hexanal, and 2-pentenal.

The more polar polymer fraction contained 21.04% of oxygen and degraded to volatile carbonyl compounds under vacuum as well as under oxygen-free nitrogen. Depolymerization and ethanolysis of these polymers were carried out in ethyl alcohol which had been adjusted to a normality of 3.5 with anhydrous hydrogen chloride. The polymeric substance formed on the filter presses of a soybean oil refinery could be depolymerized in a similar manner. It is therefore evident that oxidative polymers could be unintentionally introduced into soybean oil in various ways and serve as precursors of reversion compounds.

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Dilution Polymerization of Linseed Oil^{1, 2}

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IN thermal polymerization of fats and oils, reactions may occur between acyl groups within the same glyceride molecule or between acyl groups of different molecules. The first possibility results in a loss in unsaturation but does not increase molecular weight. Its popular name, "intra-polymerization," is therefore misleading. The second reaction, "inter-polymerization," is a genuine polymerization in that molecular enlargement results.

Because the importance of intra-glyceride reactions is still a matter of controversy, an investigation of this reaction was undertaken. Polymerization in the presence of a diluent was used on the assumption that reaction between acyl groups within the same triglyceride molecule is of lower kinetic order than reaction between triglycerides.

Materials and Methods

In this study, linseed oil was polymerized, and "heavy" white mineral oil was used as diluent.

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