

Flavor Reversion in Soybean Oil.

I. Preliminary Spectral Absorption and Isolation Studies^{1, 2}

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THE literature on flavor reversion of soybean oil to 1941 has been reviewed by Bickford (1).

During the war years apparently only one publication (2) on the subject has appeared despite the fact that this kind of flavor deterioration was of considerable concern to the fat industry as a consequence of the increased use of soybean oil.

There is more information available on flavor reversion in hydrogenated linseed oil. Lemon (3, 4) and McFarlane and co-workers (5, 6) have actively pursued this problem in recent years. Lemon (3, 4) has advanced the hypothesis that flavor reversion in hydrogenated linseed oil is apparently caused by an isomeric linoleic acid produced by hydrogenation of linolenic acid. Recently, however, it has become apparent that linoleic acid itself (7, 8) may also serve as a precursor of the iso-linoleic acids in the hydrogenation process. McFarlane, *et al.* (6), proceeding on the theory that linolenic acid is the causative agent, have recently announced the preparation of a non-reverting linseed shortening obtained from linseed oil subjected to heat polymerization and solvent segregation.

The present investigation on flavor reversion of soybean oil has been undertaken with no *a priori* notions of the causative factors involved. It is hoped that in this way a fresh and unbiased approach to the problem can be made.

Of prime importance in the study of flavor reversion is the development of a suitable method for accelerating the reversion process. In this report various techniques for catalyzing reversion have been studied, and the changes produced in the different treatments have been examined spectroscopically and organoleptically. With the experience gained from this study isolation of the causative factors has been undertaken and the progress to date is given in this report. While the latter phase of the work is no wise completed, it is hoped that the information obtained may serve as a useful guide to others engaged in similar lines of research.

Experimental

The soybean oil employed for the present studies was a commercial alkali-refined grade.³ All experiments were conducted on portions of the same sample of oil.

Deodorization of the refined soybean oil was carried out at 1 to 3 mm. pressure and 200° C. for one hour in an apparatus described by Bailey and Feuge (9).

All solvents were subjected to purification procedures before use. Chromatographic adsorptions

were made in small columns (37 x 0.6 cm.), using Fisher's adsorption alumina.⁴ The adsorptions were made from petroleum ether, which was also used as the developing solvent. Mixtures of methanol, chloroform, and acetone were the eluting solvents.

The Beckman quartz ultraviolet spectrophotometer was used for determination of ultraviolet absorption spectra. The spectrophotometric analyses on alkali-isomerized soybean oil were carried out according to the method of Mitchell, *et al.* (10) as modified by Baldwin and Longenecker (11).

Iodine values were determined by the approved A.O.A.C. method, using Wijs reagent for a 30-minute reaction period. Peroxide values were measured by a modified Wheeler method (12) and are expressed as milliequivalents of sodium thiosulfate per kilogram of fat.

Low temperature fractional crystallization of soybean oil was accomplished by techniques now in common use (13). Where very small scale crystallizations were necessary, the micro centrifuge filter of Craig and Post (14) was used.

The panel for organoleptic detection of reverted flavors was composed of a group of five subjects who became thoroughly acquainted over a period of many months with the flavors produced in soybean oil by a variety of treatments. Whenever possible, flavor tests were made on a comparative basis and no more than two samples were compared at any one time. The subject was asked to note the presence or absence of a reverted flavor and any difference in the degree of reversion. When strongly reverted samples were compared, they were usually diluted equally with mineral oil before tasting.

Methods of Producing "Reversion" Flavors in Soybean Oil.

(a) Heat reversion. A flask containing the soybean oil was immersed in an oil bath and heated with intermittent stirring over a period of one-half hour until the temperature of the soybean oil reached 200° C. The oil sample was then rapidly cooled by means of a cold oil bath and finally by a water bath. All operations were conducted in as reproducible a manner as possible.

(b) Light reversion. For producing reversion by visible light, a 10 cc. sample of refined and deodorized soybean oil was placed in a 4-inch crystallizing dish which was then immersed in a constant temperature bath maintained at 45.5° C. The even layer of oil was exposed to a 250-watt G. E. reflector-drying lamp (15) placed 3½ inches directly above the surface of the fat. Since this infrared lamp generated intense heat, it was necessary to pass a slow stream of cold water through the thermostat to maintain a constant bath temperature. Under these conditions there was not more than a slight rise in temperature (less than 2°) of the oil over long periods of time.

Ultraviolet treatment of the oil was carried out by means of a G. E. 100-watt, B-H-4 bulb. This lamp emitted light mainly in the range 310 to 435 m μ with maximum intensity of 360 m μ . The fat sample (10 cc.) was placed in a 4-inch petri dish and exposed three inches below the mercury arc of the lamp.

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³ Supplied through the courtesy of A. A. Robinson, Standard Margarine Company.

⁴ Fisher adsorption alumina for chromatographic adsorption, Fisher Scientific Co., Pittsburgh, Pa.

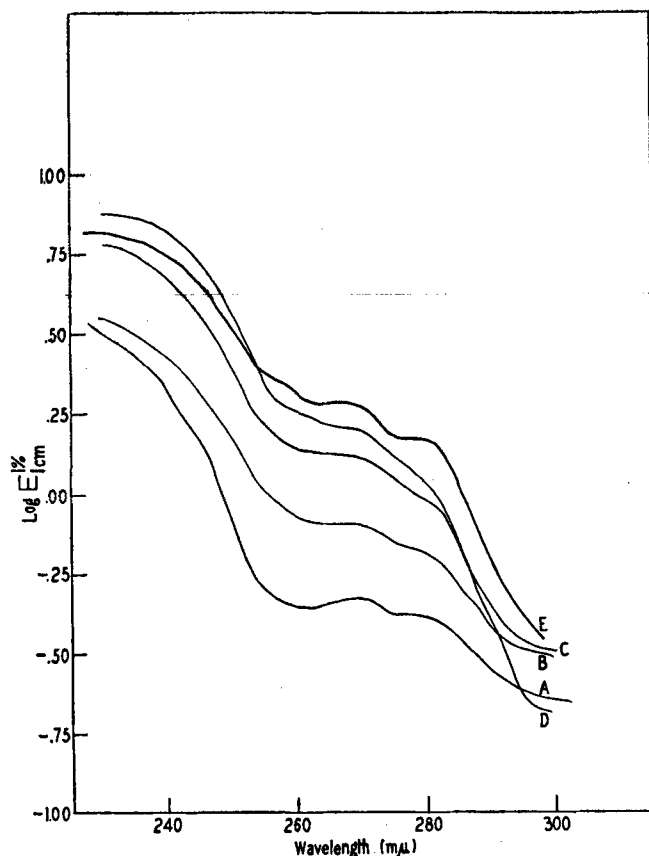


FIG. 1. Absorption Curves of Various Treated Samples of Alkali Refined Soybean Oil.

- A. Original Oil.
- B. Deodorized.
- C. Deodorized and Heat Reverted.
- D. Deodorized and Heat Reverted Six Successive Times.
- E. Stored at Room Temperature for Six Months.

Results and Discussion

Changes in the Ultraviolet Absorption Spectrum of Soybean Oil Produced by Various Treatments

The absorption curves given in Figure 1 show the spectral changes produced in alkali-refined soybean oil by deodorization, heat reversion, repeated deodorization and heat reversion, and shelf storage. Each of these treatments causes an increase in the absorption in the region below 285 $m\mu$. Inflections in the vicinity of 270 $m\mu$ are present in all of the samples, but maxima at 234 $m\mu$, characteristic of diene conjugation, are absent. Both the alkali-refined and shelf-stored oils show the fine structure in the region of 270-280 $m\mu$ that is indicative of triene conjugation. These characteristic peaks nearly disappear upon deodorization and heat treatment, yet the general absorption in the 270-280 $m\mu$ region increases. A similar behavior in the heat treatment of linseed oil was noted by Bradley and Richardson (16) who attributed it to the formation of cyclic dimers which have only general absorption in this region. Another possible explanation for the presence of inflections in the vicinity of 270 $m\mu$ is the formation of unsaturated carbonyl compounds (17).

The effect of irradiation of alkali-refined soybean oil with visible and ultraviolet light is shown in Figure 2. The samples exposed to visible light are found to have maxima in the region of 234 $m\mu$, indicating the formation of conjugated diene substances. Irradiation with ultraviolet enhanced only the general

absorption in the region of 234 $m\mu$. The effect of visible and ultraviolet irradiation on absorption in the vicinity of 270 $m\mu$ is small and probably not significant. This is in marked contrast to the results obtained by heat reversion where pronounced increases in general absorption were found to occur in the 270 $m\mu$ region. Probably this is indicative of more deep-seated changes in heat reversion than in light reversion.

Both techniques of reverting soybean oil produced characteristic flavor changes. The heat-reverted oil was usually described as fishy or painty. The flavor of the ultraviolet-irradiated oil was more difficult to describe and was characterized by a drying sensation in the roof or back of the mouth. The visible light-reverted samples were by far the easiest to recognize. They were always described as grassy or hay-like and left little or no aftertaste. These flavors were found to become more pronounced with increasing lengths of exposure.

Among the several techniques examined for catalyzing reversion the procedure employing the G. E. reflector-drying lamp has a number of advantages over the other methods. These may be enumerated as follows: (a) The reversion catalysis is rapid. In most instances it can be accomplished in a period of approximately one-half hour. (b) The oil can be

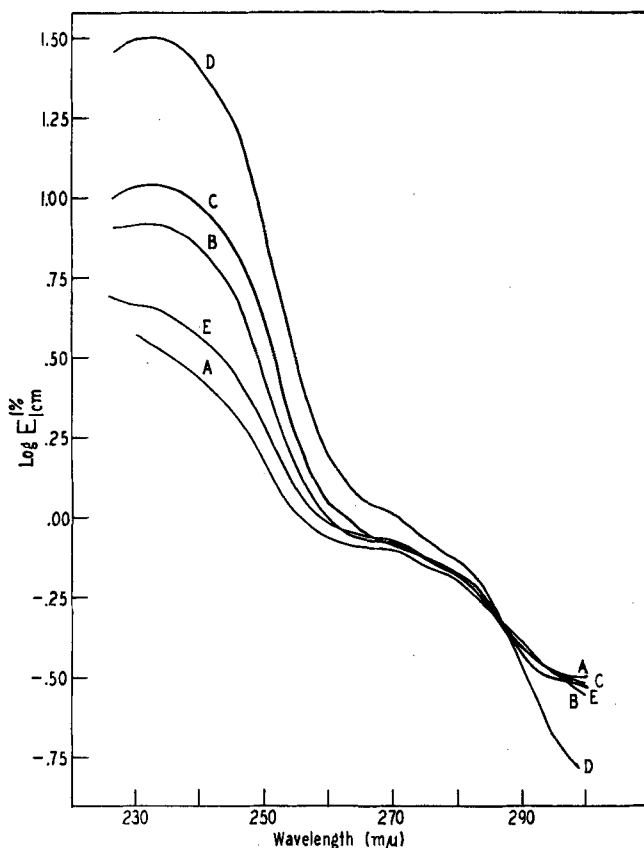


FIG. 2. Absorption Curves of Refined and Deodorized Soybean Oil After Exposure to Visible and Ultraviolet Radiation.

- A. Original Oil.
- B. Exposed Four Hours to Visible Light (G. E. R-40 Reflector Drying Lamp).
- C. Exposed Eight Hours to Visible Light (G. E. R-40 Reflector Drying Lamp).
- D. Exposed Sixteen Hours to Visible Light (G. E. R-40 Reflector Drying Lamp).
- E. Exposed Two Hours to Ultraviolet Light (G. E. B-H-4 Lamp).

reverted unaccompanied by the uncontrollable factors introduced by heating the oil at high temperatures. (c) The flavor test of the oil after reversion is distinctly characteristic and produces similar taste sensations in different individuals.

In the hope that flavor changes produced by light could be correlated with some measurable property of the oil, a study was made of the changes in ultraviolet absorption and peroxide values of the oil with increasing exposure times. The results are given in Table I wherein the $E_{1\text{cm}}^{1\%}$ values at 234 and 270 $m\mu$

TABLE I
Effect of Exposure of Soybean Oil to Visible Radiation

Exposure Time <i>hrs.</i>	Organo- leptic Test	Peroxide Value	$E_{1\text{cm.}}^{1\%}$ 234 $m\mu$	$E_{1\text{cm.}}^{1\%}$ 270 $m\mu$
0.....	fresh	0.1	3.33	0.80
1.....	reverted	8.5	4.34	0.72
2.....	reverted	21.2	5.93	0.89
4.....	reverted	39.7	8.29	0.83
8.....	reverted	89.8	11.30	0.84
16.....	rancid	196.0	31.20	1.04

are tabulated for the different exposure periods. It will be noted that the peroxides increased in a nearly linear manner and that organoleptic rancidity appeared between the 8th and 16th hour of exposure of the particular sample of the soybean oil used. The absorption at 234 $m\mu$ progressively increased during the reversion process, but the absorption at 270 $m\mu$ showed relatively little change until the fat became rancid. Although at first glance there may appear to be a correlation here among peroxide value, the selective absorption at 234 $m\mu$, and the degree of reversion, such a conclusion is not justified because of the number of variables involved. It is not clear, for example, what relation, if any, exists between the degree of reversion and rate of autoxidation. Studies on this problem are in progress and will be reported in a later communication.

Isolation of Substances Imparting Off-flavors to Soybean Oil.

The off-flavors present in reverted soybean oil can be removed readily by steam deodorization at 200° C. and 1-3 mm. pressure. It was found that when the condensates obtained from deodorization of heat and light-reverted oils were added back to the deodorized oils, the reconstituted products were described as having fishy or painty and grassy flavors, respectively. Although it is not wholly certain that these flavors are identical with those initially present, nonetheless the observations indicate that the substances causing the reverted flavor may be removed unchanged at least in part, by steam distillation *in vacuo*.

The precursors of the substances imparting the off-flavors are present in much higher concentration than the flavor-producing substances which may be designated for convenience as "reversion" compounds. This is shown by the fact that the soybean oil may be repeatedly reverted and deodorized with no apparent decrease in its tendency to revert.

Advantage was taken of this behavior to obtain a concentrate of the "reversion" compounds. A sample (200 g.) of alkali-refined soybean oil was subjected to six successive operations of heat reversion and deodorization. Spectrophotometric analysis of the fat after

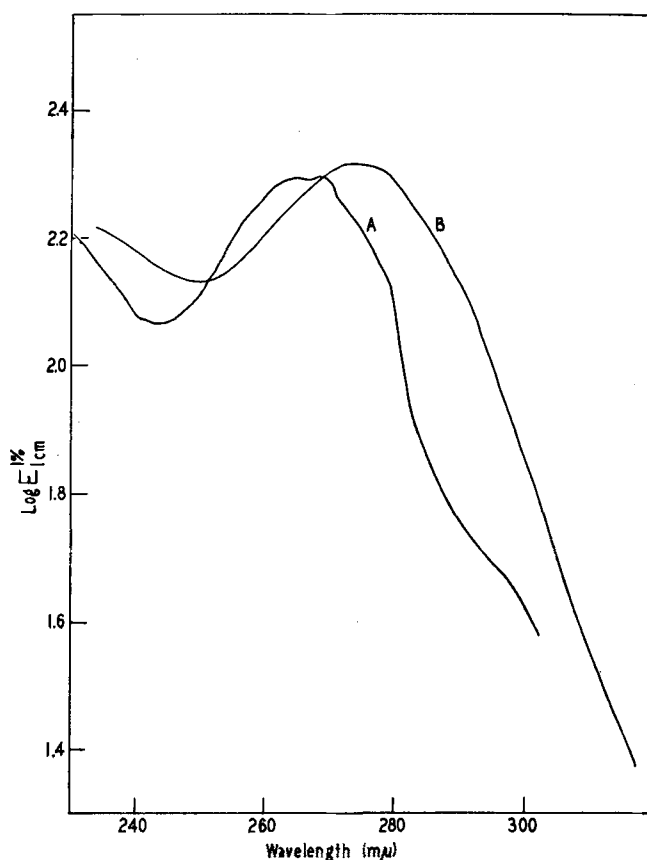


Fig. 3. Absorption Curves of Condensate.
A. In Isooctane.
B. In Alcohol.

this treatment showed no significant alteration in the fatty acid content. The condensates from each deodorization were combined and extracted with ether. The residue from the ether-extract weighed 213 mg. The aqueous phase which contained only a trace of material imparted no flavor to bland soybean oil. On the assumption that an equal amount of material was removed in each deodorization, the concentration of volatile material produced by each heat reversion was 0.017% of the oil.

The ether-soluble fraction of the condensate was found to exhibit selective absorption in the ultraviolet region between 245-280 $m\mu$ with a bicuspid peak between 264-268 $m\mu$ ($E_{1\text{cm.}}^{1\%} = 197$) when measured in isooctane, and a maximum at 275 $m\mu$ in alcohol solution (Fig. 3).

In oxidized fats selective absorption in the region of 270 $m\mu$ has been attributed to two causes (17), the resonance of unsaturated carbonyl compounds, and the presence of triene conjugated acids. These two classes of compounds may be differentiated by their response to alkali. The absorption of the carbonyl compounds is markedly increased by alkali (enolization) whereas the triene substances are unaffected. Neither of these behaviors was observed when the condensate was treated with alcoholic alkali; instead, the maximum at 275 $m\mu$ disappeared and a new and lower maximum appeared at 365 $m\mu$ with concomitant increase in general absorption in the longer wave lengths of the ultraviolet region.

Hickman (18) has shown that deodorizer scum obtained in the commercial refining of soybean and

other vegetable oils contains tocopherols, tocoquinones, sterols, and a number of other substances. The spectral properties of our deodorizer condensate seemed to indicate the presence of tocoquinone. Further experiments, given below, tend to confirm its presence.

A sterol fraction was isolated from the condensate in about 20% crude yield by low temperature crystallization (-20°) from acetone solution. After repeated crystallization from acetone the odorless crystalline material melted at $132-135^{\circ}$ C. and gave a positive Liebermann-Burchard test. Kraybill, *et al.* (19) have reported the melting point of the purified mixed phytosterols of soybean oil as $134-136^{\circ}$. The elementary microanalysis⁵ of the isolated crystalline fraction (dried at 100°) is as follows: C, 82.66%; H, 11.55%. The carbon value is lower than expected for a mixture of only sitosterol and stigmasterol and possibly may be due to retention of solvent of crystallization. Such an effect has been encountered before in the elementary analysis of sitosterol (20).

The material remaining after removal of the sterol fraction gave positive Emmerie-Engel (21) and Furter-Meyer tests (22), indicating the possible presence of tocopherol. Examination of the ultraviolet spectrum at this point showed that the maximum bicuspid peak at $264-268$ $m\mu$ was still present. The material was then chromatographed on activated alumina. Two zones were obtained: a broad brown zone near the top and below it a narrower yellow zone.

The top zone was found to consist of waxy and oily material. The waxy substance was isolated and gave a negative Liebermann-Buchard test. Not enough material was available for further examination. When the material present in this zone was subjected to alkali isomerization, charring and the deposition of insoluble material occurred. Ultraviolet examination after this treatment showed the presence of a maximum at 234 $m\mu$ but none at 270 $m\mu$. This observation would indicate the absence of linolenic acid but the possible presence of a dienoic acid.

The selective absorption shown by the condensate initially was also observed in the material present in the lower zone. This material was heated in dioxane solution containing stannous chloride and concentrated HCl according to the procedure of Tishler and Wendler (23) for cyclizing tocoquinone to tocopherol. The product obtained in this way was purified by chromatographic adsorption on alumina. It possessed antioxygenic activity in lard (antioxidant index 2 at 90° C.) and showed a maximum at 285 $m\mu$ when its ultraviolet spectrum was measured in isooctane solution. Pure tocopherol exhibits a maximum in the region of 290 $m\mu$ (24). However, in the presence of fatty materials as shown by Drummond and co-workers (25) and also observed in this laboratory, the maximum is shifted to lower wave lengths.

The sterol fraction and the material obtained from all sections of the chromatographic column and percolate were examined for the presence of substances which would impart reverted flavors to bland soybean oil. The proportions of materials used for this reconstitution were adjusted to that estimated to occur in the original reverted oil. None of the fractions examined imparted a definite reverted flavor to the soy-

bean oil. The tocoquinone fraction produced a nutty flavor, but the other fractions imparted slight if any off-flavors to the oil.

This disappearance of the "reversion" compounds during the isolation procedure suggested that they might be volatile compounds. This was found to be the case. Two aliquots of an ether extract of the condensate were evaporated on the water pump. The evaporation of one of the aliquots was continued for several hours on a hy-vac pump. Fresh soybean oil was added to both fractions. No off-flavor was imparted to the oil by the sample evacuated at low pressure but a pronounced fishy and painty flavor was given to the oil by the aliquot evaporated only on the water aspirator. Further work is in progress to isolate larger amounts of the volatile substances and to ascertain their identity.

Low Temperature Fractional Crystallization of Soybean Oil

It is apparent from the work described above that the substances producing off-flavors in reverted soybean oil are present in extremely small amounts whereas their precursors are much more abundant. Although the task of isolating these precursors seems scarcely a feasible one in the absence of a quantitative or even semi-quantitative method of assay, an attempt has been made to effect a concentration of these substances by fractional crystallization of refined and deodorized soybean oil from acetone. Bull and Wheeler (26) have reported that soybean oil can be separated into saturated and unsaturated fractions by low temperature solvent crystallization from acetone.

The systematic fractionation that was carried out in this laboratory was more extensive and is represented schematically in Figure 4. The fractionation was begun with a sample of about 200 g. of soybean oil dissolved in sufficient acetone to make a 10% solution by weight. Each of the subsequent crystalliza-

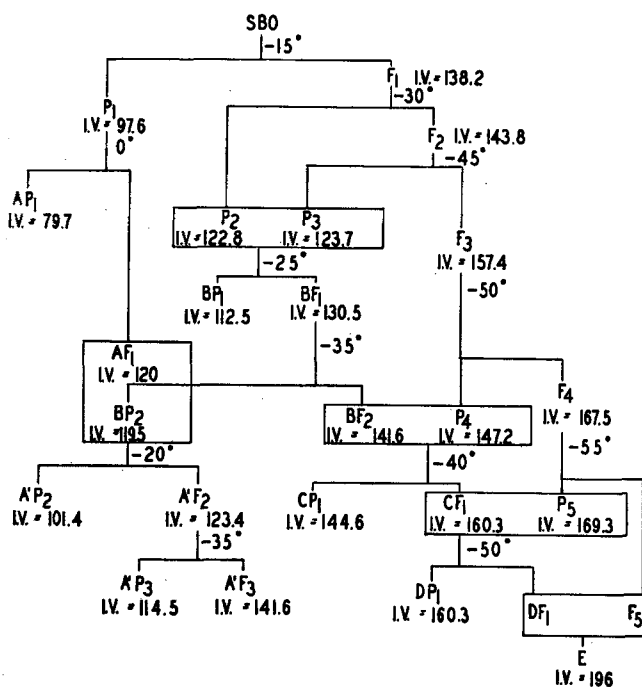


FIG. 4. Low Temperature Fractionation of Soybean Oil.

⁵ The microchemical analysis was performed by the Microchemical Laboratory, California Institute of Technology, Pasadena, California.

tions shown in the chart was performed in 10% solution, adjustments in the volume of filtrate being made where necessary. The time allowed for crystallization at each of the specified temperatures was generally two hours. By this means the soybean oil with an initial iodine value of 134 was separated into eight fractions ranging in iodine values from 80 to 196. A spectrophotometric analysis of each fraction was made for the linoleic and linolenic acid content of the component glycerides. A summary of the results are given in Table II. It may be noted that an

TABLE II
Analysis of Fractionated Soybean Oil

Fraction	Weight <i>g.</i>	Iodine Value	Linolenic	Linoleic	Oleic	Saturated
			Acid	Acid	Acid	Acids
			%	%	%	%
Original oil...	216.0	133.5	10.3	53.5	9.3	26.9
AP ₁	8.1	79.7	6.5	31.1	6.2	56.2
A'P ₂	7.1	101.4	6.9	40.1	11.0	42.0
BP ₁	43.3	112.5	7.4	44.9	11.8	35.9
A'P ₃	16.7	114.5	6.9	44.2	17.4	31.5
A'F ₃	8.2	141.6	10.7	55.2	13.8	20.3
CP ₁	60.7	146.6	10.3	58.2	14.5	17.0
DP ₁	36.1	160.3	12.8	60.7	17.1	9.4
E.....	2.0	196.0*

* Corrected for the unsaponifiable fraction. The high content of unsaponifiable matter (25%) in this fraction makes the spectrophotometric determination of the unsaturated acids uncertain.

appreciable fractionation of the component glycerides has been effected. Riemenschneider, *et al.* (27) have recently demonstrated how data of this type may be used in determination of the glyceride structures present in a fat.

The fractions obtained in highest yield, BP₁, CP₁, and DP₁ (Fig. 4) containing 7.4%, 10.3%, and 12.8% linolenic acid, respectively, were exposed to the G. E. R-40 lamp for one hour and compared in flavor with a sample of the original oil treated in like manner. The three fractions, initially bland or nearly so, and the original oil developed the grassy or hay-like flavor characteristic of reversion. No definite difference in degree of reversion was noted between the original oil and fractions BP₁ and CP₁; on the other hand, fraction DP₁ appeared to be more strongly reverted than the control. No clear-cut conclusions, however, can be drawn from these results because the tests are complicated by the fact that autoxidation in the isolated fractions proceeds at a very much faster rate than in the original soybean oil, doubtless due to the removal of natural antioxidants. It is not yet known whether variation in autoxidation rate, keeping all other factors constant, will influence the development of reversion.

The ultraviolet spectra of all the final fractions in Figure 4 were examined for the presence of preformed conjugation. Maxima in the vicinity of 234 m μ and 268 m μ , indicative of diene and triene conju-

gation, were absent. The fraction E showed a maximum at 285 m μ , apparently due to tocopherol. The unsaponifiable portion of this fraction, after removal of sterols, exhibited a maximum at 288 m μ . Surprisingly, the least soluble fractions AP₁, A'P₂, A'P₃, and A'F₃, showed maxima at 280 m μ . The substance producing this selective absorption is not known.

Summary

The ultraviolet spectral changes produced in soybean oil by treatment with heat and visible and ultraviolet light have been determined. Heat treatment appears to cause more deep-seated changes than does exposure to visible or ultraviolet irradiation. Distinct flavor changes occur in soybean oil under these different conditions; that produced by visible light of strong intensity being the easiest to recognize.

A preliminary study is reported on the methods used to concentrate and isolate the substances responsible for the off-flavor of heat-treated soybean oil.

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