Flavor Stability of Soybean Oil. 1. The Role of the Non-Saponifiable Fraction

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THERE appear to be at least two schools of thought on every phase of the soybean reversion (flavor instability) problem, and the question of the precursor of the undesirable odors and flavors is no exception. Although it seems to be generally agreed that these flavors result from the additive effect or interaction of two or more precursors, as indicated in a recent review by Bailey (1), opinion differs regarding the nature of the primary factor.

At the present time the most popular hypothesis is that the flavor instability of hydrogenated oils, at least, is due primarily to the presence of an isomer of linoleic acid, which has been called "isolinoleic" acid by Lemon (2). In his work Lemon found that the incidence of reversion in hydrogenated linseed oil could be correlated with the presence of "isolinoleic" acid (9,15-octadecadienoic acid), which he postulated was produced by the selective hydrogenation of the 12-13 double bond of linolenic acid.

Although there have been some indications that certain non-glyceride constituents, especially nitrogenous compounds, may be involved in flavor instability, Bailey (1) suggested that in hydrogenated oils reversion "may be simply a product of the glycerides" and "certainly, if non-glyceride materials are invariably involved . . . these materials are not in the unsaponifiable fraction."

In the experiments to be reported here we have extracted the soaps of hydrogenated soybean oil with petroleum ether and have found that the addition of the resulting extract of unsaponifiable material to cottonseed or peanut oils caused the typical odor and flavor of soybean reversion to develop in these oils.

Experimental

Extraction of Non-saponifiable Material. The same general conditions of saponification and extraction were used throughout all the experiments. One-kilogram samples of oil were saponified, using 400 gms. of 85% KOH dissolved in about 1.3 liters of a commercial alcohol. To insure complete saponification the reaction mixtures were heated at steam bath temperature for about three hours. After dilution with 6 liters of water the mixtures were then ready for extraction. It might be mentioned at this point that the above proportions of reagents were arrived at by trial and error. Several other proportions which were tried were unsuccessful due to emulsion formation, soap precipitation, etc.

Each of the soap solutions was extracted by one of two methods: batch or continuous. In the batch method the soaps were extracted by shaking with petroleum ether in large separatory funnels. The extracts were then washed thoroughly to remove all soaps. As would be expected, considerable difficulty was encountered due to emulsions, this difficulty undoubtedly resulting in some reduction in the yield of non-saponifiable material.

For the continuous extraction each soap solution was put into a 12-liter flask which was then filled to capacity by the addition of petroleum ether. The solution was then continuously extracted, according to the method described by Hilditch (3), for three or four 8-hour days. The yield of non-saponifiable material obtained by the latter method was considerably greater than that obtained by the batch method, but required a disproportionately greater amount of time and effort.

The Non-Saponifiable Material from Hydrogenated Soybean Oil. In all the experiments to be reported here the soybean oil was hydrogenated about 12 to 15 iodine units. A typical analysis of one of the hydrogenated oils is shown in Table I.

TABLE I

Typical Analysis of Soybean Oil Before and After Hydrogenation

	Original Soybean Oil	Hydrogenated Oil
Analytical Constant		
Iodine No.	133.9	120.7
Thiocyanogen No.	87.5	85.0
Saponification No.	194.0	194.2
Spectrophotometric Analysis		
Linoleic Acid	54	31
Linolenic Acid	8.0	4.6
Conjugated Diene	0.18	1.66
Conjugated Triene	0.03	0.016

The yield of non-saponifiable material by the continuous extraction was between .06 and .13% of the original hydrogenated oil. There appeared to be a tendency for the oils of greater flavor stability to yield less non-saponifiable material. The yields by the batch method were from .02 to .06%. As pointed out above, there was a variable loss in the batch method due to emulsion-formation.

The total yield of non-saponifiable material (0.6 gms.) from one batch of oil was dissolved in 110 gms. of refined cottonseed oil. Ten gms. of the solution were used for spectrographic analysis and the remaining 100 further diluted with 400 gms. of the same cottonseed oil. Another total extract (1.3 gms.) from a hydrogenated oil of considerably less flavor stability than that used immediately above was dissolved in the same cottonseed in an identical fashion. The two oil solutions were then carefully steam deodorized along with a portion of the untreated oil as a control. The three deodorized oils were stored in the dark at 95°F. in loosely covered wide-mouth jars and were examined at intervals for odor and flavor. The examination was purely subjective, depending entirely upon the ability of several members of our Laboratory to grade oils according to their organoleptic characteristics. (It is probably significant to note that several of these men are able to base their opinions upon 20 or more years of experience at grading edible oils.)

After five days' storage at 95°F. the control could be identified on the basis of both odor and flavor, and after the sixth day the two oils containing non-saponifiable material were characterized unanimously as having typical soybean reversion. The oil to which the second portion of non-saponifiable material (1.3 gms.) had been added was consistently judged to be the poorest of the three.

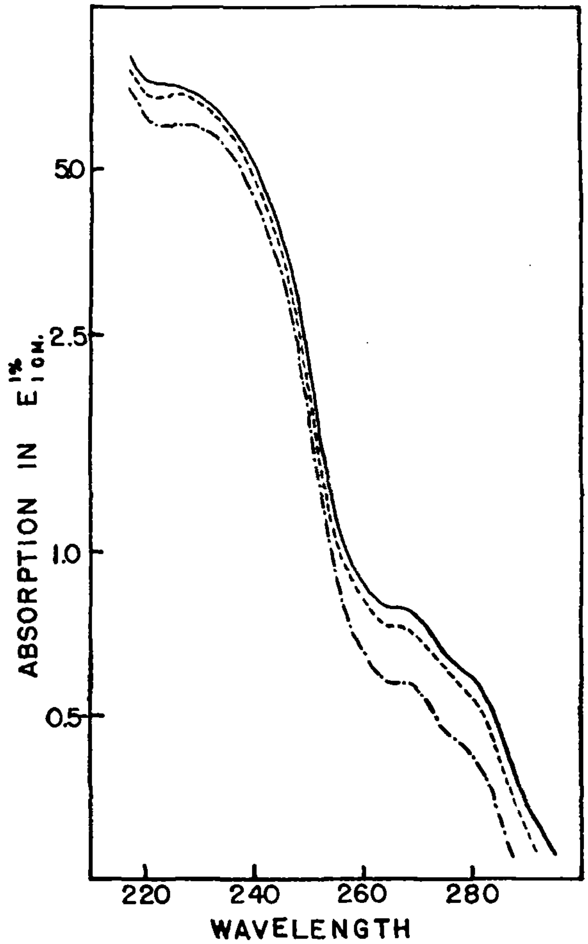

FIG. 1. Ultraviolet absorption curves for cottonseed oil and the same oil after the addition of non-saponifiable material from hydrogenated soybean oil.

— - — Cottonseed oil.
- - - - CSO with non-sap from hyd. SBO No. 1.
——— CSO with non-sap from hyd. SBO No. 2.

The spectrographic curves for the three above oils are shown in Figure 1. It will be noted that the addition of the non-saponifiable material did not alter the general shape of the absorption curve but did increase the general absorption throughout. The ultraviolet absorption curve of a portion of the non-saponifiable material from one of the hydrogenated oils is shown in Figure 2. This curve shows no characteristic peaks but merely a general absorption which is typical of a great number of different types of compounds. Consequently this lends no clue to the identity of the components of the non-saponifiable material. It is hoped that more information may be forthcoming as the result of infra-red absorption studies planned for the near future.

As a check on the above experiments with cottonseed oil, the non-saponifiable material extracted from 1½ batches of hydrogenated soybean oil was dissolved in 400 gms. of refined peanut oil. This oil and a sample of untreated peanut oil were each deodorized six hours at 220°C. At the end of this time the control was entirely odorless and tasteless, but the treated sample still had a very slight off flavor which was typical of soybean oil reversion.

After three days' storage at 95°F. the control was still odorless and tasteless, whereas the test sample had developed a slightly reverted odor and a distinctly reverted flavor typical of soybean oil and having a characteristic after-taste. After five days the control was still odorless and nearly tasteless, with no signs of typical soybean reversion. Again, the test sample had a slightly reverted odor and flavor characteristic of reverted bean oil. It did not appear, however, to be the total bean flavor but rather one of the component flavors; that is, it was more musty than grassy, with a typical after-taste.

What was probably our most interesting observation was made when the unsaponifiable material from hardened oil was added to mineral oil. The non-saponifiable from 75% of a single batch of oil was dissolved in 400 grams of mineral oil. This solution and a sample of the original mineral oil were deodorized 4½ hours at 170-180°C. When fresh, both oils were entirely odorless and tasteless. After 11 days at 95°F. both oils were still odorless and tasteless. On the 14th day, the control was still entirely bland but the mineral oil containing non-saponifiable material had developed a very unusual odor and flavor which were variously described as sweet, syrupy, maple sugar, and watermelon-like. Even after 40 days, the control oil was still entirely odorless and tasteless but the sweet odor and flavor of the treated oil remained. The flavor is very difficult to characterize but at times leaves the impression that it is one of the underlying component flavors found in reverted soybean oil. It is not very persistent and leaves but little after-taste.

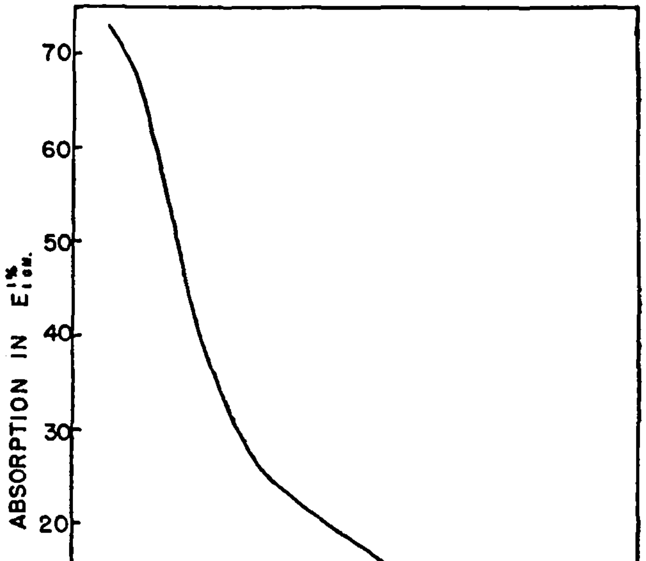

FIG. 2. Ultraviolet absorption curve for the non-saponifiable material extracted from hydrogenated soybean oil.

The Non-Saponifiable Material of Linseed Oil. Assuming that the non-saponifiable material is a precursor of reversion, it was deduced that linseed oil should yield larger quantities which could be used for further study. Consequently, a kilogram batch of refined linseed oil was saponified and extracted batchwise. As was anticipated, a rather large yield of non-saponifiable material resulted, the weight being 1.6 grams as compared with .6 grams which was the maximum yield ever obtained from hydrogenated soybean oil. The ultraviolet absorption curve of the linseed oil non-saponifiable material is similar in general character to that above for hardened bean oil, although the over-all intensity of absorption is less.

One-third of the extract from linseed oil was dissolved in 500 grams of peanut oil and the solution was deodorized along with an untreated peanut oil. After 5 days at 95°F. both oils had satisfactory odors and flavors. After 7 days, each had an off-flavor but there was no apparent difference between the two. After 12 days the two oils were off flavor to about the same degree, although the control seemed to taste somewhat on the side of rancidity and the treated oil tended somewhat toward a reverted flavor. There was very little difference, however. This observation is to be compared with that above where peanut oil containing the non-saponifiable material from hydrogenated soybean oil developed a characteristic flavor within 3 days. It should be recalled that in this experiment the non-saponifiable material was obtained from an unhydrogenated linseed oil.

Selective Adsorption Studies. Inasmuch as the components of the non-saponifiable fraction of oils are probably preferentially adsorbed in favor of the glycerides, it was decided to find out whether selective adsorption might produce an improved soybean oil. The adsorption column used (1.25 inches inside diameter) was packed with a 7-inch section of a 50/50 mixture of diatomaceous earth and powdered sugar, a 6-inch section of a mixture of diatomaceous earth and activated carbon, and at the bottom a 21-inch section of activated alumina specially prepared for selective adsorption work. After the column was wetted with petroleum ether, a solution of 500 grams of hydrogenated soybean oil dissolved in 1.5 liters of petroleum ether was percolated down through the adsorbent, the entire operation requiring about 6 hours.

The percolate was practically colorless. To increase the yield of oil, petroleum ether was allowed to percolate down through the column for 8 hours without the aid of suction. The residual oil from the two percolates was still nearly colorless and represented about 80% of the original oil. Little or no change occurred in the poly-unsaturated fatty acid composition of the oil as a result of selective adsorption, as seen in Table II. It would be presumed from Table II that any isolinoleic acid present in the hydrogenated oil was also present in the percolate.

The percolate and a sample of the original oil were deodorized under identical conditions. The fresh percolate was odorless and had very little flavor, there being a slight characteristic nutty flavor detected as an after-taste, but not while in the mouth. The hydrogenated oil which had been used for this experiment appeared to have been an exceptionally poor sample inasmuch as it reverted almost immediately in both odor and flavor after removal from the deodorizer. After one day at 95°F. the control oil had a very badly reverted odor and flavor, whereas after 3 days no change was observed in the percolate. It was still odorless, practically tasteless, but had the same after-taste as immediately following deodorization, being rather nutty but not persistent nor particularly objectionable. After 6 days, the percolate still had a good odor but a slightly reverted flavor, with some after-taste which was not as persistent nor as marked as is usual in soybean oil. This flavor might possibly be that resulting primarily from poly-unsaturated acids or their decomposition products.

TABLE II

Polyunsaturated Acids in a Hydrogenated Soybean Oil Before and After Selective Adsorption

Fatty Acids	Original Hydrogenated Oil	Percolate
Linoleic Acid	31.7	32.5
Linolenic Acid	3.3	3.1
Diene Conjugation	1.06	0.93
Triene Conjugation	.005	.007

The ultraviolet absorption curve for the percolate and the original oil showed that something had been removed which reduced the general absorption. This is as would be expected from foregoing experiments.

A 500-gram sample of an unhydrogenated soybean oil dissolved in 1.5 liters of petroleum ether was percolated down through a column identical with that above. Again the yield of oil was about 80%. The percolate and a portion of the original oil were each deodorized. After 5 days at 95°F. both samples had a slight odor and flavor, the percolate being slightly the better of the two. After 8 days the percolate had a reverted odor and flavor which was quite apparent but not too bad. The control was slightly worse but the difference was in degree only and not nearly so great as the differences noted with the hydrogenated oil above.

Discussion

The above experiments seem to establish the role of the non-saponifiable fraction as a precursor of the characteristic reverted odors and flavors of soybean oil. Unfortunately, however, the observations tend to confuse rather than clarify the over-all picture. For example, what is the explanation for the difference in the reactivity of the non-saponifiable material extracted from unhydrogenated oil and that extracted from hydrogenated oil? Certainly it would appear that that from the hydrogenated oil is more readily adsorbed and is either more easily extracted or else more unstable than the other. Thus a hardened oil was more readily improved by selective adsorption than was the unhardened oil, and the non-saponifiable material from unhardened linseed oil failed to produce the degree of typical reversion in peanut oil that was caused by an extract from hydrogenated soybean oil. Quite obviously, this point alone needs further investigation in order to establish the validity of the observation and to get an insight into the mechanism of the action of hydrogenation on the non-saponifiable fraction of oils. Work in this direction is under way in our laboratories at this time.

Another point of interest which bears repeating and further investigation is the unusual odor and flavor observed when the non-saponifiable material of hardened soybean oil was dissolved in mineral oil. It is possible that this sweet syrupy flavor is the true uncomplicated flavor resulting from the unadulterated non-saponifiable matter, and that the flavor considered to be characteristic of hydrogenated soybean oil results from the modifying action of polyunsaturated fatty acids or their decomposition products, either as an additive effect or as a result of chemical interaction.

We believe that it should be pointed out again that the selective adsorption of hydrogenated soybean oil resulted in a markedly improved oil without any apparent alteration in the poly-unsaturated fatty acid composition of the oil. In addition, both unhydrogenated cottonseed oil and unhydrogenated peanut oil developed odors and flavors characteristic of reverted soybean oil when they contained a minor proportion of the non-saponifiable material extracted from a hydrogenated soybean oil. These observations cast doubt upon the possibility that the so-called "isolinoleic" acid (9,15-isomer of linoleic acid) is a precursor of reversion, although it does not entirely rule it out. The tendency to minimize the probable effect of such a compound is given added impetus by recent publications (4, 5) which show that iso-linoleic acid is relatively unreactive both to hydrogenation and to oxidation, its activity being of the order of that of oleic acid rather than of linoleic acid. There is nothing in the structure of the compound which would indicate that it should have an abnormal odor or flavor. To the contrary, it would appear that the distance between the two double bonds would cause them to react like isolated double bonds and the compound would consequently resemble oleic acid in its various characteristics.

From a standpoint of chemical reactivity, it would seem more logical to suggest that other isomers, such as the conjugated isomers, might play a significant role. Comparing the analysis in Table I with an analysis by Bailey (4) for a similarly hydrogenated soybean oil it becomes evident that this oil contains more conjugated diene acids than it does isolinoleic acid. In the evidence presented by Lemon (2) in favor of the postulate that isolinoleic acid was a precursor of reversion, there appears to be nothing to indicate a choice of the iso-acids in preference to the conjugated acids. Although his data do not show analyses for conjugated acids it is safely assumed that they were present in all of his iso-linoleic acid concentrates. We repeat, however, that the 9-15-isomer may show abnormal reactions, but the possibility seems rather remote. It has been previously shown, on the other hand, that one of the mechanisms of fat decomposition is the formation of conjugated double bonds which appear to lead to ring formation (6, 7). The formation of such ring type compounds, either by polymerization or by interaction between the conjugated system and the non-saponifiable material would, in all probability, result in compounds of abnormal odors and flavors.

The nature of the compounds in the non-saponifiable fractions which are responsible for their flavor characteristics is obscure. There is the possibility that it may be a sterol or some of the natural hydrocarbons. There is one possibility, although somewhat improbable, we have not as yet ruled out of the picture entirely; that compound is cephalin. It has been reported that the German workers believe the phospholipids to be primarily responsible for reversion, and experiments in the removal of phospholipids by repeated water washing have shown some promise (8). Another shred of evidence pointing to cephalin is the report that the latter is primarily responsible for the off flavor in dried eggs (9). Relatively little is known about the chemical reactivity of cephalin, but Schonfeld (10) states that it is difficultly saponified, which would indicate that it may still remain in a refined oil. In addition, it has been found to be even more sensitive to oxidation than the polyunsaturated fatty acids (11). For these reasons we are making an attempt in our laboratory to determine whether or not cephalin plays a part in the flavor instability of soybean oil.

Summary

It has been found that the addition of the non-saponifiable extract of hydrogenated soybean oil to either refined cottonseed oil or refined peanut oil caused these oils to develop odors and flavors characteristic of reverted soybean oil. The non-saponifiable material from linseed oil did not produce a similar effect. When the non-saponifiable extract of hydrogenated soybean oil was added to mineral oil, a sweet, syrupy odor and flavor developed. By selective absorbents it was possible to produce a much greater improvement in hydrogenated than in unhydrogenated soybean oil. These observations are discussed in terms of their relationship to the various theories on the mechanism of reversion.

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