The Flavor Problem of Soybean Oil. VI. Flavor and Oxidative Stability of Furfural-Fractionated Oil[1]

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COUNTERCURRENT furfural extraction of soybean oil has been in commercial operation for some time for producing both a paint oil and an edible oil fraction (1). However no organoleptic or stability evaluations of the various oil fractions produced in the liquid-liquid extraction of soybean oil have been published.

Evaluations of the refined fractionated oils produced in the pilot-plant furfural extraction column in our laboratory are presented, and methods for improving the oxidative and flavor stability of the raffinate, or edible oil fraction, are described.

Experimental Procedure

The crude degummed soybean oil used in these studies was fractionated in the pilot plant liquid-liquid extraction column. This column is 47 feet high and 2.4 inches in diameter. The furfural feed is at the top, the oil feed at the middle. The heptane for the reflux feed (b.p. 88-98°C.) is introduced at the bottom of the column.

Following fractionation, the solvents were stripped from the samples, and the samples then given identical refining procedures. The first of these was the degumming step with 10% water at 65°C. for 1 hour, followed by centrifuging. The second step was alkali refining with 12°Bé. alkali (0.1% excess sodium hydroxide) and a second centrifuging. The third step, bleaching, was done at 97°C. for one-half hour with 4% of a bleach mix consisting of 15 parts of Fuller's earth and one part of Darco G-60. The physical and chemical analyses of a typically fractionated oil are given in Table I.

Deodorizations were made under identical conditions in our all-glass laboratory deodorizer (3). They were conducted for 3 hours at 210°C. under a pressure of approximately 1.0 mm. Hg and with a steam rate of 1% per hour. When stabilizers were added to the oil, the addition was made prior to deodorization. The stability of these samples was evaluated in accordance with the organoleptic testing procedure previously described (4). The amount of oil retained from any single pilot-plant fractionation was later found insufficient for the several taste panel evaluations; consequently a number of different lots of oil were used in making these studies.

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TABLE I

Chemical and Physical Constants of Furfural-Fractionated and Refined Soybean Oil

	Original Unfractionated Oil 161A	Extract 163A	Raffinate 162A
% Fractionation	.....	62	38
Iodine number (Wijs)	135.4	151.5	108.0
Linolenic Acid %	8.2	11.2	3.0
Linoleic Acid %	55.6	62.8	43.3
% Free Fatty Acid, crude	0.31	0.18	0.20
A.O.M. (8 hours)	17.6	20.9	97.0
% Ash (refined samples)	0.0002	0.0005	0.0007
% Phosphorus			
Crude degummed sample	0.014	0.0007	0.013
Refined sample	0.0004	0.0002	0.0004
Lovibond red	4.89	1.93	3.26
Lovibond yellow	35	15	20
Tocopherol (mg. per kg.)	2124	2020	225

Experimental Results

Raffinate fractions with iodine values of 105 to 110, and a linolenic acid content which varied from 2.1 to 3.2% were used in these evaluations. The different evaluations are in essential agreement with each other, and it is believed that these small differences in composition were not sufficient as variables to influence the results. The concentration effects of linolenic acid and the flavor responses as recorded by taste-panel results will not be discussed in this paper. A forthcoming paper based on the role of linolenic acid in flavor stability will present further data with detailed discussion.

TABLE II

Flavor and Oxidative Stability of Furfural-Fractionated Soybean Oil

Original Unfractionated Oil 161A	Raffinate 162A	Extract 163A	Significant Difference[1]
Flavor Score at 0 Time			
8.4 (0.31)[2]	8.7 (0.26)	8.6 (0.45)	+
After 6 Days' Storage at 60°C.			
5.2 (2.68)	5.5 (8.89)		+
6.2 (2.40)		5.7 (2.39)	+
	5.4 (8.53)	5.0 (2.64)	+
Peroxide Values (A.O.M. Conditions—8 hours)			
12.5	82.9	13.6	

[1] + No significant difference.
* Significant difference (5% level).
** Highly significant difference (1% level).
[2] Peroxide value at time of organoleptic evaluations, shown in parentheses.

In the first experiment the oxidative and flavor stability of the raffinate and extract samples were compared along with a control sample of the original unfractionated oil. The results listed in Table II show that the raffinate has the lowest oxidative stability, but the taste panel could not detect any difference in flavor stability. A study of the flavor descriptions of the stored samples did show some interesting and significant results. Of special notice is the relatively high response of painty, grassy, and melony flavors in both the extract sample and the original unfractionated oil while the raffinate re-

sponses were largely rancid. A repeat experiment confirmed the results of the first experiment. Again the flavor responses for the extract fraction and the original unfractionated oil were typical off-flavors of aged soybean oil whereas the raffinate sample developed rancidity. The relatively low oxidative stability of the raffinate was a bit surprising at first, but this was accounted for after the determination of the tocopherol content by the Emmerie-Engel procedure. The results, listed in Table I, indicate that the tocopherols are concentrated largely in the extract sample although the total amount recovered in the oil fractions was only 63% of that present in the original oil. This difference can be explained either as a loss from oxidation or as a loss to the by-product fraction (obtained as a result of back washing the extract solution).

Satisfactory stabilization of the raffinate appears to be the principal problem for successful utilization of this fraction as an edible oil. The stabilizing action of phosphatides has been known for some time (5). It has been suggested recently that the improved stability afforded by them, in part, is due to the metal scavenging effect of the fat-soluble phosphoric acid (6, 7, 8, 9). Hence an experiment was undertaken in which 0.02% of soybean phosphatides was added to a raffinate sample. The phosphatides were prepared from a crude solvent-extracted soybean oil. The oil was steamed and the hydrated phosphatides dissolved in ether. The ether was partially evaporated, and the phosphatides precipitated with acetone. In one experiment 0.02% of phosphatides was added to a raffinate fraction, and this sample was compared with an untreated extract fraction and with a sample of the original unfractionated oil. It is apparent that the flavor score of the treated raffinate was significantly higher than that of either the original oil or the extract, as shown in Table III. Other evaluations of phosphatide-treated raffinates are shown in Tables IV, VI, and VII. Perhaps the most significant information lies in the flavor analyses of the stored samples. The raffinate sample was again relatively free of the soybean off-flavors which characterized the original oil and the extract sample. Listed at the bottom of the table are the peroxide values (10) of the samples after being held under the conditions of the Active Oxygen Method for 8 hours. It is essential with this method, of course, that the oils under test be in the autoxidation phase at 8 hours so that the peroxide values will be indicative of the relative rate of oxidation.

TABLE III

Effect of Phosphatides Upon the Stabilization of the Raffinate [1]

Original Unfractionated Oil 178A	Extract 179A	Raffinate + 0.02% Phosphatides 180A	Significant Difference
Flavor Score at 0 Time			
6.7 (0.24)	6.9 (0.29)	7.2 (0.68)	+
After 5 Days' Storage at 60°C.			
5.7 (4.47)	6.0 (3.63)		+
5.2 (4.78)		7.5 (1.06)	**
	5.6 (2.97)	7.3 (0.80)	**
Peroxide Values (A.O.M. Conditions—8 Hours)			
4.5	6.0	11.3	

[1] For explanation of symbols, see Table II.

TABLE IV

Effect of Phosphatides Upon the Stabilization of the Raffinate and the Original Unfractionated Oil [1]

Raffinate + 0.02% Phosphatides	186A Raffinate	Original Unfractionated Oil + 0.02% Phosphatides	Original Unfractionated Oil 187A	Significant Difference
Flavor Score at 0 Time				
8.7 (0.07)	8.4 (0.05)	8.7 (0.05)	8.6 (0.01)	+
After 4 Days' Storage at 60°C.				
6.9 (0.88)	5.8 (3.66)			*
6.6 (0.87)			6.6 (1.20)	+
	5.9 (3.77)	7.4 (0.70)		**
6.6 (0.84)		6.9 (0.70)		+
	6.2 (3.63)		6.5 (1.01)	+
		7.5 (0.70)	6.8 (1.07)	+
Peroxide Values (A.O.M. Conditions—8 Hours)				
13.1	35.3	1.7	15.0	

[1] For explanation of symbols, see Table II.

A further study was made to determine the effect of the addition of phosphatides to the original oil and also to the raffinate sample. The oxidative data in Table IV show that the addition of phosphatides improves both the original oil and the raffinate. The flavor data show a significant difference between the raffinate sample and the raffinate sample to which phosphatides had been added. A highly significant difference was found between the untreated raffinate and the phosphatide-treated original oil. Although an organoleptic improvement was noted in these samples, no significant difference was observed between the original oil and the original oil containing added phosphatides.

TABLE V

Effect of Citric Acid and α-Tocopherol Upon the Stabilization of the Raffinate [1]

0.01% α-Tocopherol	0.01% Citric Acid	α-Tocopherol + Citric Acid	186B Control	Significant Difference
Flavor Score at 0 Time				
8.6 (0.15)	8.9 (0.03)	9.1 (0.16)	9.1 (0.13)	+
After 3⅔ Days' Storage at 60°C.				
7.7 (3.71)	8.0 (0.89)			+
7.1 (3.80)			6.4 (3.92)	+
	7.2 (1.00)	7.8 (1.45)		+
7.3 (3.63)		7.8 (1.26)		+
	7.7 (0.81)		5.5 (3.84)	**
		7.9 (1.26)	6.1 (3.99)	**
Peroxide Value (A.O.M. Conditions—8 Hours)				
12.1	20.8	7.2	37.3	

[1] For explanation of symbols, see Table II.

The effect of the additions of citric acid and of α-tocopherol was also studied in conjunction with the addition of phosphatides. The first combination of four samples prepared was: a) 0.01% α-tocopherol, b) 0.01% citric acid, c) 0.01% α-tocopherol plus 0.01% citric acid, and d) control sample. The α-tocopherol was obtained from the Eastman Kodak Company.[3] The additions were made, as usual, prior to deodorization. The oxidative data listed in Table V illustrate the effect of citric acid and α-tocopherol upon the stability of the raffinate fraction. Flavor evaluation showed highly significant differences between the citric acid sample and the control, and

[3] The name of the company is furnished for your convenience and does not imply the Department's endorsement of its product.

TABLE VI

Effect of Citric Acid and Phosphatides Upon the Stabilization of the Raffinate [1]

0.02% Phosphatides	0.01% Citric Acid	0.02% Phosphatides + 0.01% Citric Acid	219A Control	Significant Difference
Flavor Score at 0 Time				
8.9 (0.15)	9.1 (0.35)	8.8 (0.35)	8.8 (0.35)	+
After 3⅔ Days' Storage at 60°C.				
6.5 (1.10)	7.6 (0.96)			+
7.3 (0.45)			7.3 (2.48)	+
	7.9 (0.68)	7.0 (0.91)		+
7.3 (0.86)		7.3 (0.91)		+
	8.1 (0.76)		7.3 (2.18)	+
		7.3 (0.66)	7.1 (2.05)	+
Peroxide Values (A.O.M. Conditions—8 Hours)				
12.3	13.5	12.2	20.2	

[1] For explanation of symbols, see Table II.

between the combined citric acid α-tocopherol sample and the control, but no difference between the α-tocopherol sample and the control. The second combination studied was that of citric acid and phosphatides. The same procedure was followed as in the preceding experiment except that 0.02% of phosphatides and 0.01% citric acid were added. From the data of Table VI no synergism is apparent. However the combination of phosphatides and α-tocopherol did have a synergistic effect as shown by the oxidative data in Table VII.

Ash, Phosphorus, and Trace Metal Content

The effect of trace metals upon the stability of vegetable oils has been recognized for some time, but very little data have been presented on the concentration of various metals in refined oils. One difficulty has been the lack of a reliable method for the determination of trace elements. However the recent development of an improved spectrographic method for the analysis has made it possible to study the metal content in relation to stability. Present experiments indicate that a rough correlation exists. The ash, phosphorus, iron, and copper content of some furfural-fractionated oils have been determined. From the data of Table VIII it may be concluded that careful refining procedures reduce the ash content to less than 10 p.p.m. In addition, the data indicate that the fractionation process yields an extract fraction relatively free of phosphorus. The results listed for the iron and copper contents are preliminary and incomplete, but somewhat interesting and perhaps even suggestive. In the three fractionations examined, the highest concentration of copper found in the raffinate is not significant. The concentration of iron in the raffinate appears equal or lower than that of the unfractionated oil.

TABLE VII

Effect of Phosphatides and α-Tocopherol Upon the Stabilization of the Raffinate [1]

0.01% α-Tocopherol	0.02% Phosphatides	α-Tocopherol + Phosphatides	231A Control	Significant Difference
Flavor Score at 0 Time				
9.0 (0.41)	8.8 (0.35)	8.8 (0.30)	9.0 (0.35)	+
After 4 Days' Storage at 60°C.				
6.5 (4.73)	5.7 (3.41)			+
5.4 (4.51)			4.8 (7.12)	+
	5.8 (3.27)	7.2 (2.84)		*
5.7 (4.74)		6.9 (2.86)		*
	6.3 (3.21)		4.3 (7.13)	**
		6.7 (2.76)	4.3 (7.21)	**
Peroxide Values (A.O.M. Conditions—8 Hours)				
29.9	115.6	10.8	220	

[1] For explanation of symbols, see Table II.

Discussion

The furfural fractionation process offers samples with a wide range of iodine values and a corresponding variation in the amount of linolenic acid. In addition, fractions relatively free of natural pigments, antioxidants, unsaponifiable constituents, break materials, and possibly trace metals are available. These various fractions offer basic oils upon which numerous investigations and evaluations can be made of the several theories expounded to account for flavor instability of soybean oil. One of the earliest theories attributes reversion to linolenic acid; another to the unsaponifiables; and a third to phospholipids. This last has also been stressed by reports on German processing procedures. It is recognized that these materials tend to separate into the various oil fractions and their effects on oil stability should be proportionally increased or decreased in ratio to their concentration. Gloyer (1, 2) has recently discussed the fractionation of soybean oil and the properties of its various fractions. He showed that the phospholipids are concentrated in the raffinate (edible) fraction while the free fatty acids, pigments, tocopherols, and unsaponifiables are largely removed from the raffinate. Another feature of the process is the avoidance of the alkali refining step, which aids materially in the economics of the process, and also eliminates a step which many feel may materially decrease the stability of the oil.

Since the fractionation process removes tocopherols to such a large extent from the raffinate fraction, it is desirable to add back some stabilizer to the oil. Citric acid and phosphatides are believed to function in part as metal scavengers while α-tocopherol is a true antioxidant. By functioning as metal scavengers these compounds, through removal of the pro-oxidant metals, would allow the antioxidants to function much more efficiently and remain effective over a longer period. This concept could account for the observed synergistic and/or additive effect of both phosphatides and citric acid upon α-tocopherol and yet explain the lack of any additive effect of a phosphatide-citric acid combination.

Perhaps the most significant data are in the flavor responses of the furfural-fractionated samples. The raffinates in general did not develop soybean off-flavors during storage as did the extract and original unfractionated samples. This observation suggests the removal of the reversion agent during fractionation. Since it is known that fractionation removes to a large extent the tocopherols, unsaponifiables, and highly unsaturated components from the oil, there is the indication that one of these may be responsible for flavor reversion. That the addition of α-tocopherol back to the raffinate did not give rise to reverted flavors may perhaps eliminate α-tocopherol as the causative agent. The present data are not complete enough to designate whether the unsaponifiables or linolenic acid is responsible. The theory that phosphatides are the agents of reversion is weakened since the phospha-

tides are concentrated largely in the raffinate fraction and since the addition of phosphatides back to the oil does not produce reverted flavors.

The role of trace metals in flavor reversion is still not clear. The data presented here are brief and inconclusive. However it would not be too surprising to find a relatively high metal content in the raffinate fraction. The raffinate fraction has been shown to be high in phosphorus content, and it is conceivable that the phosphatides could carry metal complexes to the raffinate. During the alkali refining of the raffinate it is possible that some of the phosphatide metal complex is broken down and the phosphatides are removed, leaving detectable amounts of metal in the oil. It is believed that the observed improvement imparted by citric acid and phosphatides upon the raffinate is most easily explained as a complexing of trace metals.

TABLE VIII

Ash, Phosphorus, Iron, and Copper Content of Furfural-Fractionated Soybean Oils

	% Ash	% P	p.p.m. Fe	p.p.m. Cu
Sample 161				
Original crude degummed	.....	.0140	.....	.....
Raffinate crude	.....	.0130	.....	.....
Extract crude	.....	.0007	.....	.....
Original refined	.0002	.0004	.28	.020
Raffinate refined	.0007	.0004	.16	.021
Extract refined	.0005	.0002	.41	.027
Sample 178				
Original crude degummed	.....	.0100	.....	.....
Raffinate crude	.....	.0360	.....	.....
Extract crude	.....	.0006	.....	.....
Original refined	.0003	.....	.17	.005
Raffinate refined	.....	.....	.14	.013
Raffinate refined + 0.02% phosphatides	.0018	.....	.15	.006
Extract refined	.0009	.....	.17	.004
Sample 187				
Original crude degummed	.0340	.0110	1.40	.040
Raffinate crude	.1190	.0340	95.50	.300
Original refined	.0006	.0003	.03	.035
Original refined + 0.02% phosphatides	.0003	.0010	.03	.051
Raffinate refined	.0010	.0002	.02	.077
Raffinate refined + 0.02% phosphatides	.0026	.0011	.02	.070

Summary

Flavor and oxidative stabilities of furfural-fractionated soybean oils have been evaluated. The raffinate fractions did not develop the off-flavors typical of soybean oil as did the extract and original oil samples. The raffinate fractions have a low resistance to oxidation, but the addition of stabilizers improved the oxidative stability. Among the stabilizers tested were phosphatides, α-tocopherol, and citric acid. Citric acid and phosphatides are believed to function in part as metal scavengers.

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The Tannin and Related Pigments in the Red Skins (Testa) of Peanut Kernels

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THE red skins (testa) represent from 2.0 to 3.5% of peanut kernels and contain tannin and related pigments which will contribute to the presence of undesirable color in the protein preparations made for specialized uses unless the skins are removed completely during initial processing. The purpose of the present communication is to report the results of some investigations on the character of the tannin and related pigments.

Kryz (1) appears to be the first worker to investigate the pigmentation in peanut skins. He extracted a reddish-brown material from the skins with hot water, alcohol, and other solvents, and described its reactions with a number of reagents. Robinson and Robinson (2) reported, on the basis of various qualitative tests, that the peanut testa is rich in leucoanthocyanin which is convertible to cyanidin. More recently Tayeau and coworkers (3, 4, 5) published several papers on the skin pigments, reporting the presence of a tannin, a phlobaphene, a "leuco-anthocyanic chromogen," and a flavanone.

Experimental

Preliminary Investigations. Two and one-half kilograms of peanut skins were extracted for 48 hours at room temperature with 25 liters of ethanol containing 1% of hydrochloric acid. The dark red extract was filtered, the filtrate was concentrated under reduced pressure to approximately 2 liters, and the concentrate was diluted with 2 volumes of water. The resulting precipitate was removed by centrifugation, dissolved in ethanol, and the solution diluted with 3 volumes of diethyl ether. The precipitate was removed by filtration, washed thoroughly with ether, and dried *in vacuo*. The dark, reddish-brown product weighed 89 g.

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