The Flavor Problem of Soybean Oil. IV. Structure of Compounds Counteracting the Effect of Prooxidant Metals[1]

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SEVERAL theories have been advanced to explain the protection which organic acids give to oils. Traces of phosphatides remaining in oils after refining have been reported as the cause of flavor instability of soybean oil and have been thought to be "inactivated" by citric acid (1). The synergistic function of organic acids with antioxidants has been widely studied (2, 3, 4, 5, 6, 7, 8, 9, 10) and generally accepted. More recently, a function of deactivation of metallic prooxidants has been proposed (11, 12). In the hope of establishing the function of citric acid and of discovering substances which might be even more effective, a series of compounds related to citric acid has been tested. As a result, certain generalizations have been made as to the structural groups required to impart the protective action. This information provides a clue to the reactions involved and presents a more fundamental understanding concerning the nature of flavor instability of soybean oil.

The Function of Citric Acid and Related Compounds. In early experiments with citric acid at this laboratory it was noticed that treated oils possessed greater oxidative stability as well as greater flavor stability than untreated oils (13, 14). This observation is in agreement with the hypothesis that citric acid might function by inactivating prooxidants rather than as a synergist. This view was strengthened by experiments with polyhydric alcohols, such as sorbitol and mannitol. It would be difficult to explain the protective action of polyhydric alcohols as "acid activators" (4), but their action could be one of complexing the prooxidants.

Oxidation appears to play a key part in flavor deterioration of soybean oil. Based on 100 concurrent determinations of flavor score and peroxide value on samples of one lot of soybean salad oil, a correlation coefficient of 0.8 was found between flavor score and the logarithm of the peroxide value. This data is shown in Fig. 1. Also, it confirms the observation of Black (15) that reversion of soybean oil occurs at a peroxide value of 2 to 3. From the data it is apparent that when the flavor has deteriorated until it is no longer acceptable (flavor score 6), the peroxide value has reached 2.5. Similarly, the peroxide values of samples held under Active Oxygen Method conditions for 8 hours, have been found to be inversely related to the flavor stability of these samples. Thus, peroxide values may serve to indicate flavor stability in a related series of samples. It should be pointed out, however, that this relationship is not as close with oils obtained from different sources.

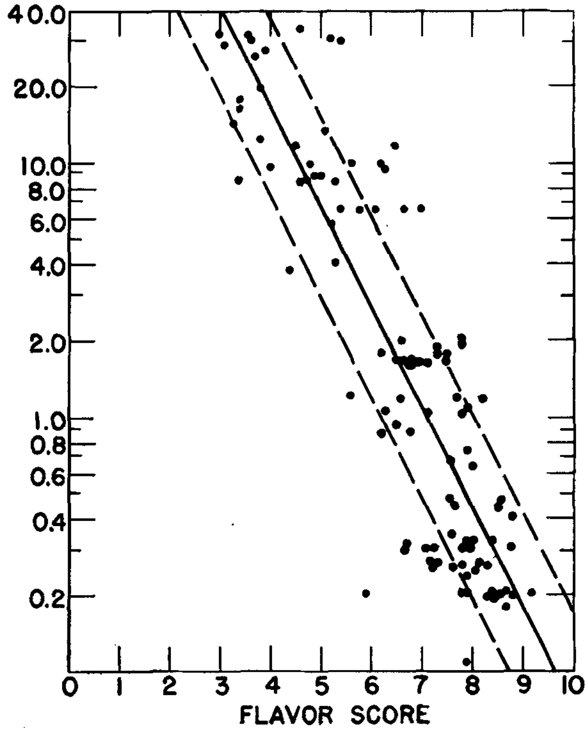

FIG. 1. Relationship of peroxide value and flavor score.

Since the most likely prooxidants were thought to be metal salts, the following experiment was conducted involving the addition of sorbitol as an iron complexing agent. To one aliquot of a refined bleached soybean oil was added 3 p.p.m. of iron as ferric chloride. To a second flask was added 0.01% of sorbitol. To a third flask was added 3 p.p.m. of iron as ferric chloride, and 0.01% of sorbitol. The fourth aliquot served as a control. All four samples were deodorized simultaneously in the four-unit glass deodorizer (16). The stability of these samples was evaluated in accordance with the organoleptic testing procedure previously described (14). Results of this experiment are given in Table I. Listed at the bottom of the table are the peroxide values (17) of the samples after being held under the conditions of the Active Oxygen Method for 8 hours. It is, of course, essential that the oils under test be in the autoxidation phase at 8 hours, in order that the peroxide values be indicative of relative rate of oxidation.

Oxidative stability and flavor stability data of Table I are in agreement in showing that sorbitol counteracts the deleterious effect of the added iron. The flavor stability of the sample to which iron and sorbitol was added is not significantly different from the sample to which only sorbitol was added. Scores of both the sorbitol treated sample and the iron-sorbitol treated sample were significantly higher than the control which, in turn, was significantly higher than the sample with the added iron. Results similar to these have been obtained in experiments in which citric acid was used as the complexing agent; however, in this instance the synergistic effect of citric acid upon naturally occurring antioxidants may also have contributed to the increase in stability.

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TABLE I

The Effect of Iron and Sorbitol on the Oxidative and Flavor Stability of Soybean Oil

Control	Iron[1] (3 p.p.m. Fe)	Sorbitol[2] (0.01%)	Sorbitol-iron	Significance[3]
		Flavor scores		
		O Time		
8.1	7.0	9.3	8.5	
		After 3 days at 60°C.		
5.6	3.6			**
5.8		7.1		*
5.8			7.1	*
	3.9		6.2	**
	4.5	8.1		**
		7.2	6.2	†
		Oxidative stability (A.O.M. conditions, 8 hours)		
33.5	62.4	10.8	18.5	

[1] $FeCl_3 \cdot 6H_2O$ (Mallinckrodt Chemical Co., Analytical Reagent).

[2] Eastman Kodak Co. (White label).

[3] Key to symbols: * Significant difference (5% level), ** highly significant difference (1% level), † no significant difference.

In order to study the complexing function of citric acid divorced from the synergistic effect, antioxidants were removed by passing a sample of soybean oil diluted with an equal volume of hexane through a chromatographic column consisting of an equal part mixture of carbon (Darco G-60) and Celite.[3] It was not possible to detect the presence of tocopherols in the oil from this column as determined by the modified Emmerie-Engel procedure (18) although some doubt exists as to the reliability of this method at low tocopherol content. After evaporation of the hexane, the sample was divided into two portions. To the first portion was added 0.3 p.p.m. of iron as iron stearate. This portion was again divided into two equal samples and to one of these 0.01% citric acid was added. The portion of oil containing no iron stearate was likewise divided into two equal samples and to one of these was added 0.01% citric acid. All four samples were then simultaneously deodorized (16) for 1 hour at 210°C.

TABLE II

The Effect of Iron and Citric Acid on the Oxidative Stability of "Tocopherol Free" Soybean Oil

Sample	Peroxide value[1] after storage at room temperature	
	After 11 days	After 22 days
Control	1.61	2.91
Citric acid[2]	0.49	0.91
Iron stearate[3]	4.46	12.8
Iron stearate + citric acid	0.40	0.87

[1] Determined on 10-gram samples.

[2] B. R. Elk and Co. (ACS).

[3] Oil and Protein Division, Northern Regional Research Laboratory.

The results of the room temperature oxidative stability test are listed in Table II. After storage for 11 days there was little difference in peroxide value between the citric acid treated samples. The difference between the control and citric acid sample could be accounted for by the presence of small amounts of metals remaining in the oil after passage through the adsorption column. There was, however, a large difference between the iron stearate sample and the iron stearate sample with citric acid. It is concluded, therefore, that the protection given this sample of "tocopherol-free" oil is due to the iron complexing function of citric acid rather than to its synergistic function with antioxidants.

A study was also made by adding prooxidant metals other than iron, namely cobalt, manganese, copper, and chromium to soybean oil. These metals were added as their chlorides in the concentrations shown in Table III. The 0.01% citric acid was added as a 20% aqueous solution and the samples were deodorized for 1 hour at 210°C. The complexing action toward the four metals by citric acid is shown by the oxidative stability data of Table III. It is interesting to note that 0.01% citric acid is insufficient to counteract 3.0 p.p.m. of added copper but is effective against 0.3 p.p.m.

Structure and Activity of Compounds Related to Citric Acid. It is doubtful if any citric acid remains as such in soybean oil when it is added at the beginning of deodorization because this acid decomposes at temperatures in excess of 150°C. The known thermal decomposition products of citric acid include aconitic and itaconic acids. These degradation products, together with compounds possessing functional groups similar to those of citric acid, were tested for protective activity by adding them to soybean oil (at concentrations of 0.01%) and deodorizing in the four-unit deodorizer along with a control sample. For purposes of tabular presentation of oxidative stability data only one control peroxide value for each of the oils is listed in Table IV. This value constitutes an average of all the control values for that particular oil. The standard deviations of means for these control samples have been calculated and are approximately ± 15%. This index variability must be taken into account in interpreting the results of the oxidative stability tests.

TABLE III

Oxidative Stabilities of Soybean Oil Containing Citric Acid and Prooxidant Metal Salts

Salt	Peroxide value (A.O.M. conditions, 8 hrs.)	
	With citric acid	Without citric acid
Control	10.7	46.6
Ferric chloride[1]—(3 p.p.m.)	125	293
Manganese chloride[2]—(3 p.p.m.)	13.1	85.4
Cobalt chloride[3]—(3 p.p.m.)	9.1	239
Chromium chloride[4]—(3 p.p.m.)	17.8	153
Copper chloride[5]—(3 p.p.m.)	291	294
Copper chloride—(0.3 p.p.m.)	44.4	288

[1,2] Mallinckrodt Chemical Co. (Analytical Reagent).

[3] Merck and Co.

[4] J. T. Baker Chemical Co. (C.P.).

[5] Harshaw Chemical Co.

From the data in Table IV certain conclusions can be drawn with considerable assurance while others must be drawn with less confidence. Since the esters and salts of citric acid are inactive, it is apparent that free carboxyl groups are essential for activity. The hydroxyl group of citric acid appears to be of little value since tricarballylic acid is of the same order of activity as citric acid. However, hydroxyl groups do appear to contribute to activity of the four-carbon dicarboxylic acids because tartaric, malic, and succinic acids form a series of acids with a decreasing number of hydroxyl groups and a corresponding de-

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[3] The use of names of firms or commercial products in this article is for product identification; it is not an endorsement of either manufacturer or product.

TABLE IV

Functionality and Oxidative Stability

Compound	Peroxide value (A.O.M. conditions, 8 hrs.)			
	Oil A	Oil B	Oil C	Oil D
Control	56.6			
Sodium citrate[1]	55.9			
Citric acid[2]	13.3			
Control		56.9		
Citric acid		16.0		
Triethyl citrate[3]		60.2		
Acetyl triethyl citrate[4]		62.8		
Tributyl citrate[5]		61.6		
Acetyl tributyl citrate[6]		63.9		
Tricarballylic acid[7]		20.2		
d-Tartaric acid[8]		12.9		
dl-Malic acid[9]		32.2		
Succinic acid[10]		35.9		
Itaconic acid[11]		25.7		
Control			34.5	
Aconitic acid[12]			27.2	
Citric acid			13.4	
Tartaric acid			14.8	
Sorbitol[13]			10.2	
With 3 p.p.m. Fe added as iron stearate				
Control				230
Succinic acid				230
2,3-butylene glycol[14]				215
Glycolic acid[15]				209
Lactic acid[16]				227

[1] Eimer & Amend.

[2] B. R. Elk & Co. (ACS).

[3] Chas Pfizer & Co. (Techn.).

[4] Chas Pfizer & Co. (Techn.).

[5] Chas Pfizer & Co. (Techn.).

[6] Chas Pfizer & Co. (Techn.).

[7] Eastman Kodak Co. (MP 159-161).

[8] Eastman Kodak Co. (MP 169-171).

[9] Eimer & Amend.

[10] Eastman Kodak Co. (MP 189-190).

[11] Fermentation Division, Northern Regional Research Laboratory (MP 163-7).

[12] Agricultural Chemical Research Division, Southern Regional Research Laboratory.

[13] Eastman Kodak Co. (White label).

[14] Fermentation Division, Northern Regional Research Laboratory.

[15] Eastman Kodak Co. (Techn.).

[16] Eastman Kodak Co. (Pract.).

crease in activity. In the presence of added iron, succinic acid appears to be entirely ineffective. Likewise, a four-carbon compound with two hydroxyl and no carboxyl groups is inactive as demonstrated with 2,3-butylene glycol. Compounds with one carboxyl and one hydroxyl group, like glycolic acid and lactic acid, also appear to be ineffective. Sorbitol, a hexahydric alcohol, is equal in activity to citric acid and tartaric acid. While compounds related to sorbitol will be discussed in the following section, it is pertinent to note here that sorbitol, citric acid, and tartaric acid are all known to form iron complexes (19, 20).

The Structure and Activity of Polyhydric Alcohols. The use of polyhydric alcohols, such as mannitol and sorbitol, for delaying the off-flavor development in soybean oil has been patented (21). Sorbitol apparently has a function similar to citric acid in complexing prooxidant metals, as is shown in the data of Table I. Experiments were performed to determine the relationship between the complexing action and the number of hydroxyl groups in the compound. From the data of Table V it appears that activity is dependent on the number of hydroxyl groups in the molecule. Sorbitol, xylitol, glycerol, and propylene glycol comprise a series of polyhydric alcohols with decreasing functionality and a corresponding decrease in activity. However, volatility during deodorization of the lower members may be a factor in their lower activity. Surprisingly, the mono fat acid esters of sorbitol and mannitol were found to be ineffective, as shown in Table V. It should be pointed out that in the preparation of these fat acid derivatives of sorbitol and mannitol, one to two molecules of water are lost, thus markedly decreasing the number of available hydroxyl groups. This explanation for the ineffectiveness of this compound appears valid on the basis of the stability data obtained for 1,4-anhydro sorbitol and 1,4-3,6-dianhydro sorbitol. Both the mono- and di-anhydro sorbitols were inactive. The dehydration and ring formation may decrease the activity in two ways: 1. Loss of hydroxyl groups and 2. introduction of steric immobility. The latter factor would seem to account for the difference in activity between sorbitol and inositol. Although both compounds are hexahydric alcohols, inositol has but a slight activity. This steric factor, as well as the availability of hydroxyl groups, may also explain the weak activity of sucrose and glucose.

The Effect of Miscellaneous Compounds Upon Oxidative Stability. In order to broaden the scope of this study a group of miscellaneous compounds was added to soybean oil (at concentrations of 0.01%) under the deodorization conditions previously described. The samples were evaluated by means of the modified Active Oxygen Method test. From the data of Table VI it is apparent that the addition of sodium cyanide to the deodorizer lowers the oxidative stability of the oil as does the addition of iron. Since the sample to which both iron and cyanide were added had a stability greater than that for either cyanide or iron alone, antagonistic or complexing action is indicated. However, the iron-cyanide sample had a much lower stability than the control sample.

TABLE V

Polyhydric Alcohols and Oxidative Stability

Compound	Peroxide value (A.O.M. conditions, 8 hrs.)		
	Oil A	Oil C	Oil D
Control	56.6		
Inositol[1]	57.3		
Sorbitol[1]	25.9		
Control		34.5	
Mannitol[1]		10.3	
Sorbitol		10.1	
Dulcitol[2]		10.5	
Xylitol[2] (crude)		17.7	
Control			45.1
Sorbitol			13.1
Sorbitan monostearate[3]			48.1
Sorbitan monooleate[3]			51.3
Sorbitan trioleate[3]			50.7
Sorbitan monolaurate[3]			44.4
Sorbitan monopalmitate[3]			43.7
With 3 p.p.m. Fe added as iron stearate			
Control			230
1,4-monoanhydrosorbitol[3]			228
1,4-3,6-dianhydrosorbitol[3]			235
1,4-3,6-dianhydromannitol[3]			243
Sorbitol			88
Xylitol (crude)			156
Glycerol[1]			192
Propylene glycol[4]			268

[1] Eastman Kodak Co. (White label).

[2] Agricultural Residues Division, Northern Regional Research Laboratory.

[3] Atlas Powder Co.

[4] Eastman Kodak Co. (BP 185-186°).

In the ethanolamine series an increase in the number of hydroxyl groups gave a corresponding increase in the oxidative stability. Diethylenetriamine and triethylenetetramine both showed improved stability over ethanolamine, which could be accounted for by the increased basicity.

Other miscellaneous compounds include Alro, a water softener and metal scavenger composed largely of the tetrasodium salt of ethylenediamine tetra-

TABLE VI

Effect of Miscellaneous Compounds Upon Oxidative Stability

Compound	Peroxide value (A.O.M. conditions, 8 hrs.)				
	Oil A	Oil C	Oil D	Oil E	Oil F
Control	56.6				
Ethanolamine[1]	53.3				
Diethanolamine[1]	45.3				
Triethanolamine[1]	35.2				
Diethylenetriamine[2]	28.2				
Triethylenetetramine[2]	23.4				
"Alro A"[3]	60.4				
Control		34.5			
Cephalin[4]		9.0			
Lecithin[4]		13.2			
Total phosphatides[4]		10.0			
Phosphoric acid[5]		8.0			
Control				16.4	
Control+Fe(3.0 p.p.m.)				96.2	
Control+NaCN(0.01%)[1]				60.3	
Control+Fe NaCN				48.0	
With 3 p.p.m. Fe added as iron stearate					
Control			230		
Lauryl gallate[6]			199		
Gallic acid[2]			191		
Nordihydroguaiaretic acid (NDGA)[7]			201		
Control					277
Hyamine 1622[8]					272
DMP-30[8]					265
Ethylene thiourea[8]					218

[1] Eastman Kodak Co. (Pract.).
[2] Eastman Kodak Co. Techn.).
[3] Alrose Chemical Co.
[4] Oil and Protein Division, Northern Regional Research Laboratory.
[5] B. R. Elk & Co. (85% reagent grade).
[6] Oil and Fat Division, Eastern Regional Research Laboratory.
[7] Nordigard Corporation.
[3] Rohm and Haas Co.

acetic acid, which was shown to be ineffective in oils. Neither D.M.P.-30, a 2,4,6-tri (dimethylaminomethyl) phenol, nor Hyamine 1622, a benzyl trimethyl ammonium chloride, were effective against 3.0 p.p.m. of iron as iron stearate. Ethylene thiourea had some activity, as indicated by its index of 218 compared with the control of 277. Commonly accepted antioxidants were found to be only slightly effective in complexing iron, as shown in the data for lauryl gallate, gallic acid, and nordihydroguaiaretic acid (N.D.G.A.).

The activity of phosphatides is of particular interest in relation to the data for phosphoric acid. Phosphoric acid has frequently been employed as a metal scavenger (11). It is highly possible that the phosphatides constitute a fat-soluble phosphoric acid and may thus function as a metal scavenger rather than as a conventional antioxidant. These data and other data presented here pose the question of whether many of the so-called synergists may not display synergistic effects in part because of their ability to complex prooxidant catalysts as well as because of their "acid activation" of "inhibitols."

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Summary

A study has been made of the effectiveness of various polycarboxylic acids and polyhydric alcohols in improving the stability of soybean oil. Certain observations have been made regarding the structural groups required and the possible mechanism of reaction. Since salts and esters of organic acids are inactive, free carboxyl groups are required. Among the four carbon atom dicarboxylic acids activity increases with the number of hydroxyl groups. Within the polyalcohols activity increases with the increase in number of hydroxyl groups. Steric immobility and loss of hydroxyl groups by dehydration reduces activity. Evidence is presented which attributes to citric acid and certain polyhydric alcohols the role of metal scavenger. For example, it has been demonstrated that the addition of citric acid and sorbitol to soybean oil containing prooxidant metallic salts effectively increases the oxidative and flavor stability of the oil. By using a sample of treated soybean oil with no detectable tocopherols, in order to eliminate synergistic effects of citric acid, it has been shown that the prooxidant effect of iron stearate is counteracted by the presence of citric acid. The demonstration that polyhydric alcohols increase the flavor and oxidative stability is compatible with their known metal complexing properties. Evidence is presented which indicates a relationship between flavor stability and oxidative stability.

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