Antioxidant Material From the Osage Orange (Bois D'Arc) Fruit[1]

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ALTHOUGH Osage orange wood and the dye contained therein have found considerable commercial application, no industrial or domestic uses have been found for the Osage orange fruit. In an earlier paper (1) the authors reported a partial analysis of the fruit and emphasized its potentialities as a source of industrial raw materials such as oil, sugars, resins, pigments, etc. This report is concerned with the discovery of a highly potent antioxidant among the constituents of the Osage orange fruit.

Introductory Remarks

Osage Orange, Bois d'Arc, Hedge Apple, and Horse Apple are common names for a tree known botanically as *Maclura pomifera* (also *Maclura aurantiaca, Toxylon pomiferum,* and *Ioxylon pomiferum*). The tree (See Figure 1), has been planted widely throughout the United States, principally for hedges, and is now found in almost every state. In recent years however there have been fewer plantings, and in fact, many hedges have been bulldozed out to recover the land so occupied. Nevertheless there are great quantities of the tree still thriving. The writer has observed areas where there are almost pure stands of Osage orange trees covering 100 or more acres.

FIG. 1. Osage orange tree.

Osage orange trees grow rapidly and under a wide range of soil and climatic conditions and will reproduce from both seeds and sprouts (2).

Osage orange fruit is produced in tremendous quantities. It is fed to a limited extent to horses, mules, and cattle. The fresh fruit contains fat-soluble aromatic materials which produce an undesirable flavor in dairy products when eaten by cows. It is unpalatable to man.

The fruit has a unique physical appearance, as may be seen from Figure 2. It is commonly referred to as the "hedge ball" and is well known because of the white sticky sap which exudes very freely when the fruit is bruised or cut. Each fruit weighs one to three pounds.

FIG. 2. Osage orange fruit.

The seed kernels, which represent 20% of the dry weight of the fruit are very tasty, especially after roasting and salting. They can be used as such in pastry, confectionery items, etc. The seed kernels contain 42% by weight, of a pale, yellow, semidrying oil. Removal of the oil leaves a meal containing 67% protein. Squirrels thrive on these seed kernels, and the squirrel hunters know that the fattest squirrels are likely to be found along the hedgerows. From the standpoint of mechanical handling and processing it is entirely feasible to accomplish a separation of the seeds from the other fruit portions.

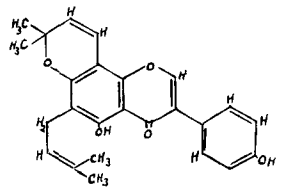
Antioxidant

The writer has observed that the dried fruit of the Osage orange contains approximately 10% by weight of a material that is remarkably effective in delaying the onset of oxidative rancidity in lard and other oleaginous substances which are subject to oxidative deterioration.

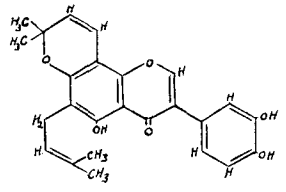
Osage orange fruit contains several different pigments. The principal ones are osajin and pomiferin, both of which are classified chemically as isoflavones. Their chemical structures have been elucidated by

[1]Presented at the Spring Meeting of the American Oil Chemists' Society at Houston, Tex., April 28-30, 1952.

Wolfrom and his associates of Ohio University (3) and are shown as follows:



OSAJIN



POMIFERIN

Pomiferin is much more effective as an antioxidant than osajin (Table I), presumably because it possesses two hydroxyl groups in the ortho-position with respect to each other whereas osajin does not. Most of the better known commercial antioxidants contain one or more pairs of functional groups (OH, OCH_3, NH_2, etc.) in the ortho (1, 2) or para (1, 4) positions.

TABLE I

Comparison of Osage Orange Antioxidant with NDGA, and G4C Antioxidants in Lard

No.	Description of samples	Final stability in hrs. AOM
1	0.1% COOA + 0.005% CA	100
2	0.05% COOA + 0.005% CA	71
3	0.005% COOA + 0.005% CA	16
4	0.05% COOA without CA	66
5	0.1% ROOA + 0.005% CA	119
6	0.05% ROOA + 0.005% CA	83
7	0.005% ROOA + 0.005% CA	20
8	0.05% ROOA without CA	74
9	0.01% Osajin	9
10	0.01% Osajin + 0.005% CA	11
11	0.1% Osajin	24
12	0.008% NDGA + 0.005% CA	79
13	0.1% G4C	64
14	0.1% BHA	72
15	Untreated lard (control)	8

CODE: COOA—crude osage orange antioxidant (pomiferin, osajin, and resinous constituents).

ROOA—refined osage orange antioxidant (pomiferin, with small amount of osajin and some resinous material).

NDGA—nordihydroguaiaretic acid.

G4C—propyl gallate with citric acid and lecithin (product of Griffith's Laboratories).

BHA—butylated hydroxyanisole.

CA—citric acid (used as synergist).

Pomiferin and osajin are both yellow crystalline compounds. Within the fruit they are mixed intimately or associated with a material which darkens to a brown color during the dehydration of the fruit. This brown, noncrystalline material becomes extracted along with the osajin and pomiferin and contributes its color to the residue obtained after removal of the solvent. Solvent fractionation gives a khaki brown mixture of crystalline and noncrystalline substances which possess high antioxidative properties. Separation of the mixture into its components gives substances which, when used separately, have lower antioxidative potencies than when used as a mixture.

The antioxidant in its most potent form appears to be a mixture of pomiferin, with a small amount of osajin, and one or more amorphous resinous substances which act synergistically with the pomiferin.

The most active concentrate obtained was then tested with several well known commercial antioxidants by means of the active-oxygen method, using the Swift stability apparatus and unprotected lard as the substrate and control.

It was considered desirable to determine what effect the temperature at which the fruit is dried has on the potency of the antioxidant obtained therefrom. A quantity of fruit was dried at 80° C. in a vacuum oven. Another quantity was dried in an air oven at 120°C. The dried fruit obtained by each of the two methods of drying was ground to 40-mesh in a Wiley mill and subjected to extraction with a number of different solvents. In each case the extract was weighed to obtain the yield and then taken up in dipropylene glycol solution in 10% concentration by weight. Samples of unpreserved lard were mixed with the various dipropylene glycol solutions of the extracts so as to give 0.1% concentration of each extract. Then AOM tests were run on the samples of lard thus treated. The results are shown in Table II.

TABLE II

Osage Orange Extracts Dissolved in Dipropylene Glycol (10% Concentration by Weight) and Added to Lard in Approximately 0.1% Concentration of Extract Based on the Weight of the Lard.

No.	Solvent used	Dried at 80°C. in vacuum oven		Dried at 120°C. in air oven	
		Percent yield	AOM stability of 0.1% in lard	Percent yield	AOM stability of 0.1% in lard
1	Acetone	29.0	38.5	25.0	42
2	Ethyl ether	23.4	50.0	23.6	53
3	Dioxane	32.2	38.5	20.4	63
4	Carbon tetrachloride	24.8	45.5	24.8	40
5	Benzene	23.6	55.0	17.4	67
6	Petroleum ether	16.0	45.5	13.0	20
7	Methanol	57.4	34.5	51.2	36
8	Ethanol	50.0	36.5	42.0	42.5
9	Isopropanol	38.0	39.0	31.0	48
10	Cold water	40.0	7.0	.....	.....

From the standpoint of establishing methods of processing of the fruit it was necessary to determine in which parts of the fruit the antioxidant is most or least concentrated. For this part of the study the fruit was separated by hand into fruit sap, fruit bulk, seeds, core, and pigments. The antioxidative potency of these separate portions of the fruit was determined by direct additions of the materials to untreated samples of lard or by using benzene or ethyl ether extracts. The results appear in Table III.

TABLE III

Distribution of Antioxidant Within the Osage Orange Fruit

No.	Description	Percentage used in lard	AOM stability in hours
1	Sap:		
	Oven dried	0.1	5.5
	Vacuum dried	0.1	6.5
2	Seeds:		
	Benzene extract	0.1	9.5
	Ethyl ether extract	0.1	10.0
3	Core:		
	Dried and ground	0.5	9.0
4	Pigment:		
	Mechanically separated	0.1	22.0
	Dried at 115°C.	0.1	29.0
5	Fiberous and pigmented portion:		
	Benzene extract	0.1	42.5
	Ethyl ether extract	0.1	42.5

A series of tests was run to determine the results attainable by use of the Osage orange antioxidant with various acids suspected as having synergistic action. The acids tried and the results obtained are shown in Table IV.

Since considerable variation in antioxidative potency occurred among the extracts reported in Table II and since none of these extracts exhibited a potency comparable to the most active preparation on hand, it appeared desirable to separate each extract into fractions and to determine the activity of the various fractions in order to ascertain what steps in refinement might be advisable. The results of anti-

oxidant studies on the fractions obtained from the ethyl ether and benzene extracts are reported in Table V. These extracts yielded 43, 48, and 12%, respectively, of petroleum ether-insoluble materials.

One of the most interesting and worthwhile observations made during this investigation involved the use of the dehydrated fruit pulp directly as an antioxidant material. Dried Osage orange fruit was ground to about 40-mesh and added to lard at about 100°C. After having been stirred a few minutes, the insoluble material was filtered off. The lard then possessed a stability approximately one-tenth that of the most active preparation. Results of these tests are shown in Table VI.

TABLE IV

AOM Stability Data on Samples of Lard Treated with Osage Orange Antioxidant (0.1%) and Various Compounds (0.005%) to Determine Their Values as Synergists

No.	Description of samples	AOM stability in hours:	
		Antioxidant + acid	Acid alone
1	Lard, untreated control	4.0	.....
2	Antioxidant (0.1%) without synergist	95.0	.....
3	Antioxidant + tartaric acid	114.5	6.0
4	Antioxidant + pyruvic acid	96.0	5.5
5	Antioxidant + phosphoric acid	104.0	6.0
6	Antioxidant + oxalic acid	114.5	6.5
7	Antioxidant + malonic acid	91.5	5.5
8	Antioxidant + l-malonic acid	107.0	5.0
9	Antioxidant + lactic acid	90.0	7.0
10	Antioxidant + fumaric acid	95.5	6.0
11	Antioxidant + citric acid	112.0	5.5
12	Antioxidant + d-isoascorbic acid	116.0	5.5
13	Antioxidant + ascorbic acid	116.5	5.5

TABLE V

Stability Data on Lard Treated with Fractions Prepared from Extracts of Osage Orange Fruit (Concentration in lard, 0.1% in each case)

No.	Description of samples	AOM stability in hours
1	Ethyl ether extract, crude	53
2	Liquid portion	33
3	Nonliquid portion	54
4	Petroleum ether-soluble portion	27.5
5	Petroleum ether-insoluble portion	101
6	Solubles and insolubles recombined	56.5
7	Benzene extract, crude	45
8	Petroleum ether-soluble portion	29
9	Petroleum ether-insoluble portion	83

TABLE VI

Antioxidant Potency of the Dried and Ground Fruit When Added to Lard Directly

No.	Description	AOM stability in hours
1	Dried fruit pulp (1.33%)	106
2	Dried fruit pulp (.67%)	70.5
3	Dried fruit pulp (.33%)	43.5
4	Benzene extracted fruit pulp (1%)	9
5	Ethyl ether extracted fruit pulp (1%)	5.5
6	Ethanol extracted fruit pulp (1%)	5
7	Lard, untreated control	4.5

Data obtained by studies on the effect of Osage orange antioxidant on the stability of fats other than unhydrogenated lard are shown in Table VII.

Discussion

A very satisfactory 83-hour lard was obtained by the use of 0.05% Osage orange antioxidant and 0.005% citric acid. Without citric acid 0.05% of the antioxidant gave a 74-hour instead of an 83-hour lard. In these same tests 0.01% NDGA with 0.005% citric acid gave a 79-hour lard, 0.1% of G4C antioxidant (a mixture of propyl gallate, lecithin, and citric acid) gave a 64-hour lard, and 0.1% BHA gave a 72-hour lard.

TABLE VII

Stability Data on Vegetable Oils and Hydrogenated Fats Containing Additions of Osage Orange Antioxidant

No.	Description of samples	AOM stability in hours
1	Soybean oil, refined, salad oil grade	10
2	Soybean oil (1) + 0.1% antioxidant	12
3	Soybean oil, laboratory deodorized	6.5
4	Soybean oil (3) + 0.1% antioxidant	11
5	Hydrogenated soybean oil	62.5
6	Hydrogenated soybean oil (5) + 0.1% antioxidant	155
7	Cottonseed oil, salad oil grade	10.5
8	Cottonseed oil (7) + 0.1% antioxidant	27.5
9	Hydrogenated cottonseed oil	42
10	Hydrogenated cottonseed oil (9) + 0.1% antioxidant	42.5
11	Hydrogenated lard	26
12	Hydrogenated lard (11) + 0.1% antioxidant	103

On a comparable basis with G4C, 0.1% of the Osage orange antioxidant gave a 119-hour lard. These data indicate that on a unit-weight basis, the Osage orange antioxidant is about one-fourth as active as NDGA and approximately twice as active as G4C or BHA.

While NDGA is the most highly potent antioxidant presently acceptable for use in foods, it suffers from an economic disadvantage because of its relatively high cost. Other commercial antioxidants are sold at less cost per unit-weight, but are also less potent. The Osage orange antioxidant has several attractive economic advantages as well as a high order of potency. There is a plentiful supply, renewable each year. The dried fruit contains an amazingly high concentration (approximately 10% by weight) of the active principals. The antioxidant is readily extracted from the fruit and refined by simple solvent fractionation procedures. After removal of the antioxidant, the remaining fruit constituents are completely utilizable either as raw materials for further processing or simply as a component for mixed feeds.

A distinct advantage is gained by direct extraction of the dried fruit with hot lard. A 60-hour lard can be obtained by treating hot lard with 0.5% of the dried and ground fruit and then filtering off the undissolved plant materials. The same advantage is attainable if it is desired to incorporate the fruit constituents with any other oleaginous materials, such as vitamin concentrates, peanut butter, etc.

The data given in Table II show that the antioxidant can be removed from the fruit by any one of a number of solvents although some prove more suitable for use than others. No solvent was found which would extract the antioxidant selectively without various amounts of extraneous substances appearing in the extract. The results in Table V show that the antioxidant is only slightly soluble in petroleum ether and that the petroleum ether-insoluble portions of the benzene and ethyl ether extracts possessed the greatest antioxidant activity. The most active form of the antioxidant reported herein was the petroleum-ether insoluble fractions of extracts obtained from the fruit with other solvents such as ethyl ether. Recrystallization by dissolving in a small volume of ethyl ether and precipitation by adding petroleum ether resulted in a somewhat higher antioxidant potency. The highest actual yield of active material was obtained by fractionation of the ethyl ether extract although ethyl ether extracted only 24% while methanol extracted as much as 57% of the fruit materials.

The Osage orange antioxidant is fairly stable to heat as is evidenced by the results shown in Table II, indicating that material dried at 120° C. in an air oven gave extracts of higher potency, in most cases,

than material dried at 80° C. in a vacuum oven. Also the antioxidant suffered no deleterious effects when heated in lard at 150° C. for a considerable time when lard extracts of the fruit were being made. This point is of considerable practical importance since it makes possible the treatment of lard with the Osage orange materials during the latter part of the rendering process. The undissolved plant material then may be filtered off with other materials following the rendering operation.

Studies are being conducted to determine the stability of the Osage orange antioxidant under conditions of deep fat frying and carry-over value into baked goods. Also studies are being followed to determine what chemical modifications of the antioxidant may be effected to improve its properties, uses, and potency.

Most of the antioxidant materials are located within the fibrous portion of the Osage orange fruit. Results reported in Table IV show that the fruit sap, seeds, and core contain only a small percentage of the antioxidant. The seed kernels, which constitute 20% of the dry fruit mass, contain 42% by weight of a pale yellow semi-drying oil. Removal of the oil leaves a meal containing 67% protein.

The Osage orange antioxidant responds well to the synergistic action of certain acids according to the results shown in Table V. Of these acids, tartaric, oxalic, malonic, citric, and ascorbic acids gave the best responses. It is of interest that these acids are either dicarboxylic or tricarboxylic. Ascorbic acid gave a slightly better result than citric acid, but the slight advantage would not justify its use because it is much more costly than citric acid.

Examination of the data in Table VII reveals that 0.1% of the Osage orange antioxidant approximately doubles the AOM stability values for cottonseed and soybean salad oils. It increases the stability of hydrogenated soybean oil 2.5 times and that of hydrogenated lard about four times. For some reason it failed to affect the stability of the samples of hydrogenated cottonseed oil tested.

It is reasonable to suppose that the Osage orange antioxidant will prove useful in such materials as oil-soluble vitamin concentrates, cosmetics, pharmaceuticals, lubricating greases, and many other substances that are subject to oxidative deterioration.

In concentrations required for antioxidant function the Osage orange antioxidant imparts no noticeable flavor or odor and is nontoxic. Large doses of the antioxidant have been fed to rats over a period of several weeks without any apparent ill effects on growth or metabolism of body tissues. Pomiferin is an isoflavone and represents a group of well known plant constituents, many of which are known to occur in plants used for foods.

Acknowledgments

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REFERENCES

1. Clopton, J. R., and Roberts, Ammaretto, J. Am. Oil Chem. Soc., *26*, 470-472 (1949).
2. Betts, H. S., "Osage Orange," Forest Service, U. S. Dept. Agric. Bull., "American Woods," 4 pp., Aug. 1945.
3. Wolfrom, M. L., Harris, W. D., Johnson, C. F., Mahan, J. E., Moffet, S. M., and Wildi, J. Am. Chem. Soc., *68*, 406-418 (1946).

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The Effect of Chlorophyll on the Color and Value of Vegetable Oils

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THE Color Committee of the American Oil Chemists' Society has worked for a number of years on the development of a spectrophotometric method for measuring oil colors. A.O.C.S. Tentative Method Cc 13C-50 revised October, 1951, is a direct outgrowth of this work. The A.O.C.S. Photometric Method has not proven acceptable to many persons because oil colors obtained by its use do not agree in all cases with oil colors obtained by the Wesson Method, A.O.C.S. Official Method Cc 13b-45 using Lovibond glasses. Disagreement between the two methods can be attributed mainly to variations in the chlorophyll content of oils. The Photometric Method just does not compensate for chlorophyll in the same amount and degree as does the Wesson Method. Figure 1 shows transmittance curves for two oils containing different amounts of chlorophyll. The chlorophyll contents of the oils, as well as A.O.C.S. colors, and Lovibond red values are shown. The disagreement between A.O.C.S. color and Lovibond red as chlorophyll changes is apparent. At low chlorophyll levels A.O.C.S. colors are slightly higher than Lovibond red values, and at high chlorophyll levels A.O.C.S. colors may be very much lower than Lovibond red values.

Effect of Chlorophyll on Apparent Bleachability of Oil. The problem of checking bleaching earth activ-

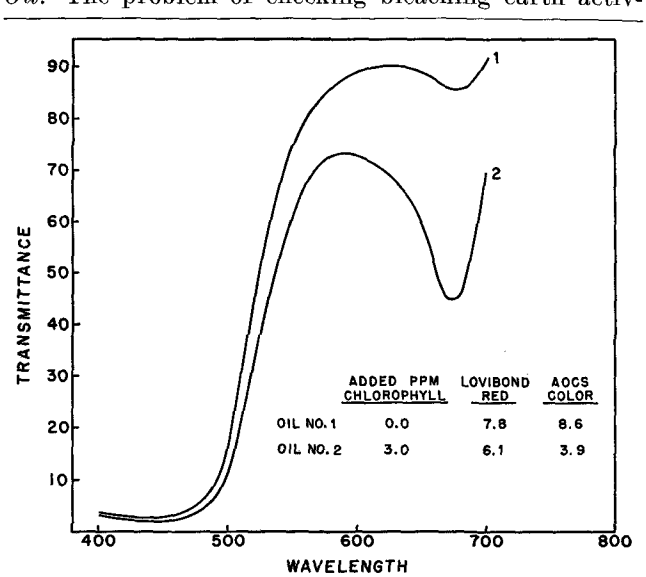

FIG. 1. Effect of chlorophyll addition on oil colors.