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The Reduction of Free Gossypol in Cottonseed by Pressure Cooking[1]

G. H. GRIBBINS,[2] Blaw-Knox Company, Pittsburgh, Pennsylvania

COTTONSEED is unique among commercial oil seeds in that it contains pigment glands. The contents of these glands are responsible for the characteristic colors of the crude oil and meal products (1) and for the toxicity of uncooked meal to farm animals such as swine and chicks (2). The principal pigment of cottonseed is gossypol. This pigment constitutes from 35 to 50% of the weight of the pigment glands and 1 to 2.5% by weight of the kernel is seed of American grown varieties (3, 4). The existence of two general forms of gossypol is recognized by the cottonseed industry, free gossypol and bound gossypol. The toxicity of cottonseed is related to the presence of free gossypol whereas bound gossypol is free gossypol which has reacted chemically to form compounds which are physiologically inert. This publication deals only with free gossypol.

According to Boatner (5), gossypol is an unstable complex polyphenolic compound which owes its preservation in cottonseed to the protection furnished by the pigment gland wall. To destroy gossypol the gland walls must therefore be ruptured. These walls are tough, only a small fraction being broken by rolling or grinding the whole meats; but on contact with moisture they are readily ruptured and the effect of moisture increases at elevated temperatures (5). High moisture, high temperature, cooking then serves as one practical method for rupturing the pigment glands and destroying gossypol.

In cooking, a number of controllable variables affect the degree of gossypol reduction, such as cooking temperature, cooking time, moisture content, particle size, and the nature of the material being cooked. To investigate these variables, cooking experiments were made on whole meats, flaked meats, and solvent extracted flakes.

Experimental Procedure

The cooking tests were made in a cast aluminum autoclave, 10 in. deep and 13 in. in diameter. A basket, 6½ in. in diameter and made of 12-mesh galvanized wire, was used to hold the sample. The basket was supported above the water level in the cooker on a tripod ring. The water was brought to boiling with the steam relief valve open, and the wire basket containing a 100-g. sample was placed in the cooker. The desired pressure was attained as rapidly as possible and held for the specified cooking time. Using a multiple gas flame, it took about 30 seconds to reach 10 p.s.i.g. and 60 seconds to reach 20 p.s.i.g. cooking pressure. The cooking times reported began after the specified pressure was reached. After cooking for the desired time, the burner was turned off and the pressure released. This took only a few seconds. The lid was then immediately opened and the sample removed. It is apparent that the longer the cooking time, the less the percentage error in its measurement.

To minimize any error in the results due to gossypol reduction in the interval between cooking and analysis, the samples were dried to approximately 12.0% moisture in a vacuum oven at a temperature less than 130° F. By running control samples, it was shown that the vacuum drying did not introduce any detectable error in the results as compared with drying in air at room temperature. One-half of each cooked and dried sample was sealed in an air-tight container and sent to a commercial laboratory for gossypol analysis. The other half of each sample was retained for moisture analysis.

All gossypol analyses were made by the A.O.C.S. Tentative Method Ba 7-49. This method determines total free gossypol and gossypol-like substances. Moistures were determined by toluene distillation [Method 27.4 of the Association of Official Agricultural Chemists (6)]. Oil contents of the samples used for cooking were found by the A.O.C.S. Official Method Ba 3-38 (7).

Laboratory Cooking Experiments

To investigate cooking variables, experiments were made at pressures ranging from 5 to 20 p.s.i.g. and times from 10 to 90 minutes. The effect of moisture was determined by varying the initial moisture of the samples from 5 to 15%, by dessication over sulfuric acid to reduce the moisture, and by careful addition of water to increase the moisture. Samples in which the initial moisture was adjusted were tempered for at least 24 hours before cooking.

Following is a description of the samples used in the cooking experiments:

SERIES A. *Unconditioned Whole Meats (Cooking Tests Made January-February 1949).* This series of experiments was made on commercially delinted, hull-free, whole cottonseed meats (No. 1586) received in November 1948, from Delta Products Company, Wilson, Ark. These whole meats were unconditioned, i.e., they received no heating or steaming treatment either in the plant or in the laboratory prior to cooking.

SERIES B. *Unconditioned Whole Meats (Cooking Tests Made April 1950).* This series of tests was also made on whole meats (No. 1586), the only difference being that Series B cooks were made after 17 months' storage compared with approximately 3 months' storage for Series A meats. The meats were stored in an air-tight container at room temperature.

SERIES C. *Unconditional Flaked Meats (Cooking Tests Made April 1950).* For these tests a sample of whole meats (No. 1586) was flaked at room temperature to 0.030 in. thickness. The flakes were made on single-pair, smooth laboratory rolls, 5½ in. diameter by 10 in. long, turning at 470 r.p.m.

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[2] Consulting Engineer, 2829 Espy Ave., Pittsburgh 16, Pa.

SERIES D. *Solvent Extracted Cottonseed Flakes (Cooking Tests Made April 1950).* Flakes prepared by commercial solvent extraction (No. 1588), received in November 1948, from Delta Products Company, Wilson, Ark., were used in Series D tests. At this plant the whole meats were cooked at atmospheric pressure in a steam jacketed cooker, flaked to 0.010 in. thickness on 20-in. diameter single-pair flaking mills, extracted for approximately 45 minutes with commercial hexane at 130° F., and desolventized in a bank of steam jacketed dryers. The sample used in the cooking tests was obtained at the discharge of these dryers.

SERIES E. *Solvent Extracted Cottonseed Flakes (Cooking Tests Made September 1949).* The flakes used in Series E cooking tests were prepared in the laboratory by solvent extraction of commercial flakes (No. 1738). The raw unextracted flakes, 0.010 in. thick, made from Mississippi Delta seed (1948 crop), were flaked at 180° F. on 13-in. diameter, single-pair, smooth rolls, turning at 257 r.p.m. They were extracted in the laboratory by immersion in commercial hexane at 120° F. for a total period of 80 minutes, then washed with fresh solvent, and desolventized in air at room temperature.

SERIES F. *Conditioned Flaked Meats (Cooking Tests Made May 1949).* For these tests, the whole meats (No. 1586) were conditioned by steaming at 0 p.s.i.g. for 3 minutes and then drying in a vacuum oven to 12.0% moisture. The conditioned meats were flaked at 140° F. to 0.030 in. thick with 5½-in. diameter, single-pair, smooth rolls.

Experimental Data and Results

Table I gives the analyses of the samples used in the cooking experiments. The gossypol content at the beginning of each cook, on an oil-free, moisture-free, gossypol-free basis, is designated as X_o.

TABLE I

Analysis of Samples Before Cooking

Series number	Crude lipids (M.F.B.)	Moisture	Free gossypol	
			As is	O.F.M.F.G.F. basis = X_o
	%	%	%	%
A	37.7	10.0	1.03	1.86
B	37.7	9.4	1.13	2.04
C	37.7	9.4	1.13	2.04
D	2.8	8.13	0.94	1.06
E	6.7	8.6	0.93	1.10
F	37.7	12.0	1.0	1.86

The experimental data and the residual gossypol analyses of the cooked samples are given in Table II. The gossypol analysis of each cooked sample was converted to an oil-free, moisture-free, and gossypol-free basis. This analysis is designated by X = (lbs. gossypol)100/lbs. O.F.M.F.G.F. meats. The cooking temperature in Table II is not necessarily the steam temperature; it is the temperature read from a chart of vapor pressure of water over cottonseed meats or extracted flakes. Only when the water content during cooking is less than 10% is there any significant difference between the temperature given and the temperature corresponding to the steam pressure. Both the initial moisture and the cooking moisture are reported. The cooking moisture is a calculated value found by adding to the initial moisture the conden-

TABLE II

Laboratory Cooking Data and Results

Cook number	Cooking pressure	Cooking temperature	Moisture content		Free gossypol after cooking O.F.M.F.G.F. basis = X	Reduction in gossypol	Cooking time	
			Initial	Cooking			Actual	Calculated by Equations 7, 8
	p.s.i.g.	°F.	%	%	%	%	mins.	mins.
Series A—Unconditioned Whole Meats								
1	15	250	10.0	17.2	1.08	41.9	10	12.0
2	15	250	10.0	17.2	0.99	46.8	20	19.8
3	15	250	10.0	17.2	0.81	56.4	30	29.1
4	15	250	10.0	17.2	0.51	72.6	60	59.3
5	15	250	5.0	12.1	1.24	33.3	20	22.4
6	15	250	8.0	15.2	1.08	41.9	20	20.2
7	15	250	12.0	19.2	0.865	53.5	20	21.5
8	15	250	15.0	22.2	0.815	56.1	20	19.2
10	7.5	235	10.0	16.5	1.41	24.2	20	19.8
11	19	257	10.0	17.5	0.76	59.1	20	20.0
Series B—Unconditioned Whole Meats								
23	15	250	9.4	16.6	1.62	20.5	5	9.8
24	15	250	9.4	16.6	1.41	30.5	15	16.2
25	15	250	9.4	16.6	1.36	33.0	15	19.0
26	15	250	9.4	16.6	1.04	48.5	40	36.5
27	15	250	9.4	16.6	1.04	48.5	40	36.5
38	19	257	9.4	16.9	1.00	50.9	30	28.2
39	19	257	9.4	16.9	0.70	65.7	50	51.5
40	5	229	9.4	16.1	1.585	22.3	30	31.1
41	5	229	9.4	16.1	1.32	35.3	60	59.1
Series C—Unconditioned Flaked Meats (0.030″ Flakes)								
28	15	250	9.4	16.6	1.67	18.1	5	
29	15	250	9.4	16.6	1.39	31.8	10	
30	15	250	9.4	16.6	1.15	43.6	25	
31	15	250	9.4	16.6	0.735	63.9	50	
32	15	250	9.4	16.6	0.715	64.9	60	
Series D—Solvent Extracted Flakes (Commercial)								
33	15	254	8.13	14.4	1.05	0.94	5	
34	15	254	8.13	14.4	0.86	19.1	15	
35	15	254	8.13	14.4	0.76	28.5	30	
36	15	254	8.13	14.4	0.71	33.2	45	
37	15	254	8.13	14.4	0.67	36.9	60	
Series E—Solvent Extracted Flakes								
20	10	245.5	8.6	14.6	0.625	43.2	30	
21	15	254	8.6	15.0	0.36	67.2	60	
22	15	254	8.6	15.0	0.26	76.3	90	
Series F—Conditioned Flaked Meats (0.030″ Flakes)								
15	15	250	10.0	17.2	0.405	78.2	20	
16	15	250	10.0	17.2	0.425	77.1	20	

sation of steam required to heat the flakes to the cooking temperature. Also included in Table II is the percentage of reduction in gossypol for each cook $(X_o - X)100/X_o$.

Rate of Disappearance of Gossypol

The rate of disappearance of gossypol is a function of cooking time, cooking pressure, and moisture content of the sample. The data of Table II will be examined to evaluate the effects of these variables.

Effect of Cooking Time on Gossypol. To demonstrate the effect of cooking time, independent of the other variables, the data of cooks 1 to 4 in Series A and all the cooks of Series D are plotted in Figure 1. It will be observed that the reduction of gossypol, rapid at first, becomes quite slow after one-half hour of cooking. The rate of gossypol reduction for the whole meats is approximately double that for the solvent extracted flakes; during a 60-minute cooking period the gossypol in the extracted flakes was reduced from 0.94 to 0.57% and from 1.0 to 0.28% in the whole meats.

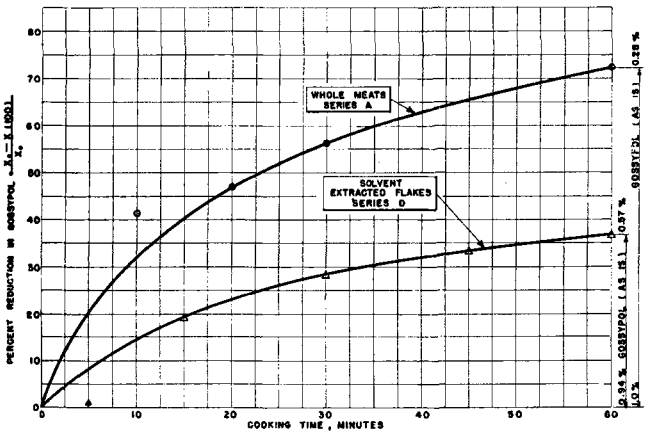

FIG. 1. Effect of pressure cooking at 15 p.s.i.g. on gossypol.

It was found that the data of Figure 1 could be replotted as 1/X versus cooking time to give straight lines. This suggested that all the data could be correlated by similar plots, as is shown in Figures 2 and 3.

Also included in Figure 3 is a plot of data obtained by the Southern Regional Research Laboratory (U.S.D.A.) on cooking raw cottonseed at a pressure of 10 to 12 p.s.i.g. and an initial moisture in the seed of 7.6% (8). For convenience of plotting the residual gossypols were corrected to an O.F.M.F. basis by assuming the cooked seed contained 18.0% oil and 7.6% moisture. Since these analyses are based on seed and not on meats, the S.R.R.L. data cannot be compared with that for Series A to F. The fact however that data of other workers could be plotted in the same manner lends additional support to this method of correlating cooking data.

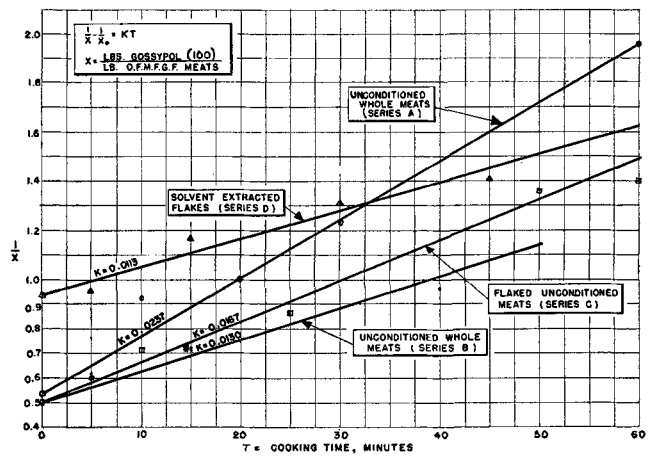

FIG. 2. Effect of pressure cooking at 15 p.s.i.g. on gossypol.

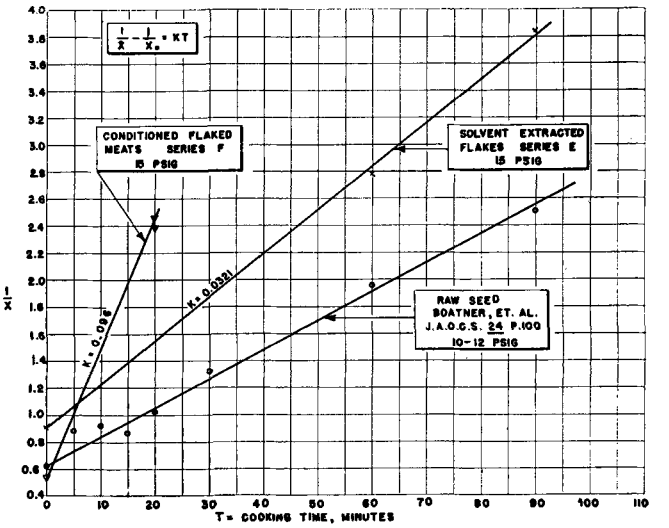

FIG. 3. Effect of pressure cooking on gossypol.

Although it is probably without theoretical significance, a straight line plot of 1/X versus time is indicative of a second order reaction mechanism. The equation for a second order reaction in which gossypol is the only reactant is:

$$dX/dT = -KX^2 \quad \ldots\ldots\ldots\ldots 1$$

Where X = gossypol remaining at any time, T
K = rate constant for the reaction

Integrating:

$$1/X - 1/X_o = KT \quad \ldots\ldots\ldots\ldots 2$$

This is the equation for a straight line of 1/X versus T, as plotted in Figures 2 and 3, where K is the slope and $1/X_o$ is the intercept on the ordinate axis.

Since the slope of the curves for Series A to F in Figures 2 and 3 are indicative of the rate of gossypol disappearance, the following broad conclusions may be drawn:

a) The rate of disappearance of gossypol during cooking decreases with an increase in the storage time of the meats. (K for Series A is about 80% greater than for Series B.)

b) The rate of gossypol disappearance is increased by flaking meats prior to cooking. (K was increased 400% by flaking meats which had been stored six months, Series F, and 30% by flaking the same meats after 17 months' storage, Series C.)

c) The slowest rate of gossypol reduction is on solvent extracted flakes. (K for Series C meats was approximately 50% greater than for Series D extracted flakes.). The absence of oil can probably account for the poor results on extracted flakes. This is shown by the higher rate constant K on Series E flakes, which contained 6.7% oil compared with Series D flakes containing 2.8% oil.

Effect of Moisture on Gossypol. By selecting cooks 1 to 8 from Series A in which the only variable was the moisture content, Figure 4 was prepared. The

value of K as determined from Equation 2 was plotted against both the initial moisture and cooking moisture. The straight lines that resulted were extrapolated to K = 0 where there is no reaction. The fact that the plot shows no disappearance of gossypol when the initial moisture is zero, despite the presence of 7.0% cooking moisture, indicated that superficial moisture condensed on the meats was without effect in destroying gossypol. Since it is known that gossypol is protected by tough walled pigment glands and that moisture is necessary to rupture these gland walls, the reduced rate of gossypol disappearance at low moisture content is not surprising. Tempered moisture, that is moisture which has had time to become thoroughly dispersed throughout the meats, appears to be necessary for the reduction of gossypol.

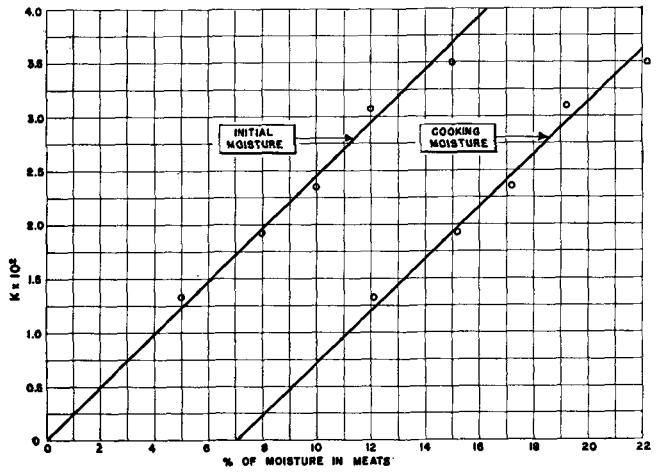

FIG. 4. Effect of moisture on gossypol in whole meats (Series A) at 15 p.s.i.g.

The straight line plot of Figure 4 suggests that K is proportional to the initial moisture content, that is:

$$K = CM \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad 3$$

Where C = a constant which is the slope of the curve

M = initial moisture in the whole meats, %

Effect of Temperature on Gossypol. By selecting cooks 1 to 4 and 10, 11 from Series A and all the cooks of Series B, in which temperature was the only variable, Figure 5 was prepared. K is plotted against temperature on semi-log paper, giving straight lines. Although the only difference in the meats of Series A and Series B was storage time, the rate constant K doubles with a 10° F. temperature rise for Series A and with a 14° F. rise for Series B meats.

The straight line plot of Figure 5 suggests that log K is a linear function of temperature, that is:

$$K = 10^{(B\Theta+E)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad 4$$

Where B = a constant which is the slope of the curve

E = a constant which is the intercept of the temperature axis when log K = 0

Θ = temperature of the meats, ° F.

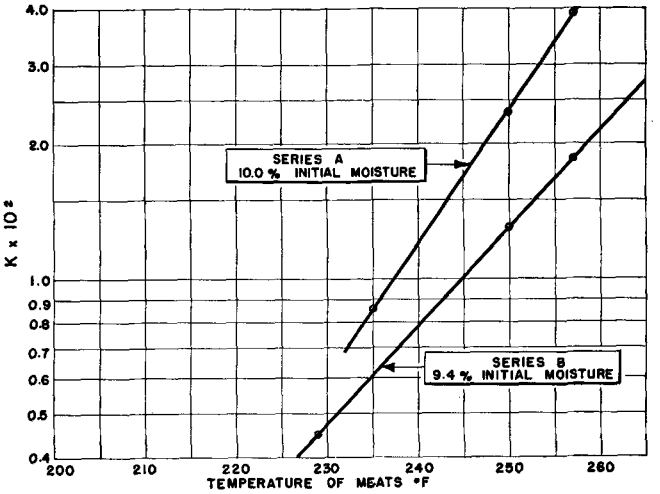

FIG. 5. Effect of temperature on gossypol in whole meats.

Correlation of Cooking Variables

Assuming that the straight lines of Figures 4 and 5 are to be expected in any cooking experiments—and there is evidence that this is the case, then a general equation can be obtained correlating the cooking variables: time, initial moisture content, and temperature, as follows:

Combining Equations 3 and 4:

$$K = M\ 10^{(B\Theta+A)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad 5$$

Where A = a new constant representing the previous constants C and E

Substituting Equation 5 into Equation 2:

$$1/X - 1/X_o = M\ 10^{(B\Theta+A)}\ T \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad 6$$

Where X = gossypol in cooked sample on O.F.M.F.G.F. basis, %

X_o = gossypol in uncooked sample on O.F.M.F.G.F. basis, %

M = initial moisture in meats, %

Θ = temperature of meats during cooking, °F.

T = cooking time, minutes

B, A = constants, the value of which are dependent on a number of factors, such as the nature of the material being cooked, particle size, history of the sample, and possibly the type of cooking apparatus

Values of the constants A and B can be found for Series A and Series B whole meats by selecting values of K and Θ from Figure 5 and substituting in Equation 5.

The complete equation relating the rate constant K with initial moisture content, time, and temperature, in Series A whole meats is then:

$$1/X - 1/X_o = M\ 10^{(0.0296\Theta - 10.02)}T \quad . \quad . \quad . \quad . \quad 7$$

The complete equation relating the rate constant K with initial moisture content, time, and temperature, in Series B whole meats is then:

$$1/X - 1/X_o = M\ 10^{(0.022\Theta - 8.363)}T \quad . \quad . \quad . \quad . \quad . \quad 8$$

These equations can be verified by substituting the experimental cooking data for Series A and Series B meats in Equations 7 and 8 and solving for the time T. A comparison between the actual cooking times and the times as calculated from these equations is Given in Table II. It will be observed that there is a reasonably close agreement between the actual and the calculated values. This indicates that the methods used to correlate the cooking variables for whole meats are valid, at least for practical purposes.

Summary

It has been demonstrated that the rate of disappearance of free gossypol during pressure cooking correlates well when using the equation of a second order chemical reaction. This equation applies to raw seed, whole meats, flaked meats, and solvent extracted flakes. For whole meats it was shown that the value of the rate constant K for the disappearance of free gossypol varies in direct proportion to the initial moisture content of the meats; and the log of K varies linearly with the temperature of the meats. Based on these factors a method has been presented by which the three cooking variables: moisture, temperature, and time, can be correlated into a single equation for any one series of cooks on cottonseed meats.

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Chemical Investigation of the New England Horse Chestnut, Aesculus hippocastanum[1]

VIVIAN B. EHLERS and G. ALBERT HILL, Wesleyan University, Middletown, Connecticut

IN the early years of the last war the shortage of oils made the investigation of hitherto unimportant sources of oil worth exploring. The fruit of the horse chestnut, *Aesculus hippocastanum*, was reported as being used in Europe as a source of oil. The genus Aesculus, because of the striking appearance and the abundance of the various species, has interested many investigators, and papers recording the results of their work have appeared since 1833 (7). Romanesi (8) in 1834 mentioned obtaining the oil by heating and pressing the nuts. Stillesen (11) in 1909 was the first person to carry out anything approximating a thorough investigation. He reported the oil as having the following constants: d_{15}^{15}, 0.9260; n_D^{20}, 1.4747; saponification value, 194.5; iodine value, 95.4; Reichert-Meissl value, 1.54; Hehner value 92.9; acetyl value, 13.5. Other investigators reporting comparable values are Kaufmann and Baltes (6), Rousset (9), Sabalitschka (10), Heiduschka and Zeilers (3), and Chaplet (1). Kaufmann and Baltes (6) in 1938 reported the acid content to be linolenic, 2.2%; linoleic, 22.7%; oleic, 67.2%; stearic, 3.6%; palmitic, 4.4%. This work has been confirmed by Chopin (2) in 1946. In this hemisphere grows another member of the genus, the American buckeye. These facts led to the initiation of work in this laboratory on nuts from these two sources. A complete chemical analysis and an investigation of feasible commercial values are to be carried out.

The chemical constants do not reveal anything unusual. The iodine number assigns the oil to the nondrying class. Fatty acids of low molecular weight are not a part of its glyceride structure. Monethenoid unsaturation apparently predominates over the polytype. The oil analyzed in this laboratory was extracted from meal of the whole nut (kernel and shell) ground to pass through a 50-mesh screen and extracted with petroleum ether (b.p. 40°-72°) for 50 hours. The yield of oil was approximately 5%. The oil appeared reddish brown by transmitted light and a deep greenish yellow by reflected light. It had a pronounced bitter taste. The following physical constants were determined: specific gravity at 20° C. 0.9047; index of refraction at 25° C. 1.4633.

The chemical constants were determined by well-established procedures (5) and are shown in Table I.

TABLE I

Chemical Constants of Horse Chestnut Oil

Saponification number	167.5
Unsaponifiable matter (%)	2.97
Total fatty acids (%)	92.4
Soluble acids (% as butyric)	1.13
Saturated acids (%)	6.67
Unsaturated acids (%)	84.73
Iodine number	103.0
Thiocyanogen number	76.0
Hehner number	89.0
Acetyl value	17.2
Acid value	14.9
Reichert Meissl number	0.00
Polenske number	1.05

Separation of the Unsaturated Acids. The oil was saponified in an alcohol solution with potassium hydroxide, and the fatty acids were converted to the lead salts by treatment of the faintly acid solution with alcoholic lead acetate. The lead salts were separated according to a modified Twitchell lead-salt-alcohol separation as given by Hilditch (4). From

[1] This work was made possible through funds provided by Wesleyan Industrial Scientific Research.