tall oil fatty-acid soap, and straight tall oil resin-acid soap were evaluated. The effect of fatty acid-resin acid ratio was determined by using mixtures of those soaps. Sodium rosinate, sodium oleate, and mixtures of these soaps were used as comparison standards. Curves plotted show wash-test data and foaming values as functions of the ratio of fatty soap to resin soap.

The data indicate in terms of detergency: a) tall oil soap has a higher value than sodium rosinate; b) sodium oleate is better than tall oil fatty-acid soap, but the latter is approximately equivalent to soaps from various unsaturated vegetable oils; c) both tall oil resin-acid soap and rosin soap have low detergency on cotton; d) the detergency of most mixtures of tall oil fatty-acid and resin-acid soaps at lower concentrations is greater than would be predicted from the individual soaps, indicating a synergistic effect.

As a rough approximation, tall oil soap without unsaponifiables is equivalent to a corresponding mixture of sodium oleate and sodium rosinate. The presence of unsaponifiables lowers both detergency and foaming. Tall oil soap is somewhat less sensitive to hard water than sodium oleate.

Significant differences between detergencies of soaps, and especially between soap mixtures, are obscured when launderometer tests are run at moderate soap concentrations. These differences are readily detected at lower concentrations.

REFERENCES

1. Hastings, R., Pollack, A., and Wafer, J. M., Soap and Sanitary Chemicals *20*, 24-27, 70 (1943).
2. Treffler, Andreas, Soap and Sanitary Chemicals *20*, 29, 30, 63 (1944).
3. Anderson, R. H., and Wheeler, D. H., Oil and Soap *22*, 137-141 (1945).
4. Browning, B. L., and Calkin, John B., Paper Trade Journal *123*, No. 26, 45-47 (1946).
5. Borglin, J. N., Mosher, P. R., Noble, Barbara, and Pushon, T. Oil and Soap *20*, 77-84 (1943).
6. Ross, John, and Miles, Gilbert D. *18*, 99-102 (1941).

[Received December 30, 1949]

Characteristics of Solvent-Extracted and Hydraulic-Pressed Okraseed Oils

S. A. HUSSAIN, State of Hyderabad, India, and F. G. DOLLEAR, Southern Regional Research Laboratory,[1] New Orleans, Louisiana

THE okra plant, *Hibiscus esculentus* L., may be grown throughout most of the cotton belt of the United States. Research has recently been directed towards breeding non-shattering varieties of okra with seed adapted to mechanical harvesting and having a higher oil content than those usually grown for edible purposes. Although okraseed is not at present a very desirable seed for oil and meal production, research may be expected to yield improved strains which may take their place with or possibly replace cottonseed as an oilseed crop in some areas of the South.

The production of okra for industrial uses has been discussed by Edwards and Miller (1). In processing this seed, the problem of decortication requires more attention since the present methods are not effective in separating the kernels from the hulls (2). Edwards and Miller (1) reported that okraseed oil could be refined, bleached, and deodorized by the usual methods without any serious problems. They found that the okraseed meal is comparable to other meals now in commercial use for feeding livestock.

Analyses of okraseed and extracted meal have been reported by Kilgore (3), Halverson and Naiman (4), Edwards and Miller (1), Markley and Dollear (2), and Clopton *et al.* (5). The characteristic properties and composition of okraseed oil reported by Jamieson and Baughman (6) and others (2, 5), have been compared with those of cottonseed and peanut oils. The reported composition of okraseed oil determined by chemical methods is approximately, 25.5-29.7% linoleic acid, 41.5-41.9% oleic acid, and 28.8-29.7% saturated acids. However the fact that discrepancies have been observed between these results and composition as determined by the spectrophotometric method has been reported by Edwards and Miller (1) and Clopton *et al.* (5). The amount of linoleic acid spectrophotometrically determined by Clopton *et al.* was 13.2% although calculation from the iodine and thiocyanogen values and calculation from iodine and saponification values of the distilled methyl esters, gave 27.1% and 26.1% linoleic acid, respectively. The reported linoleic acid content of 27.1% calculated from the reported iodine and thiocyanogen values appears to be too low, probably because of an error in the calculations. The present authors have recalculated the composition of this okraseed oil using the equations adopted by the American Oil Chemists' Society (7) and the iodine value (Wijs) and thiocyanogen values reported by Clopton *et al.* (5). When the factor 1.046 is used to convert the results from a glyceride to acid-in-oil basis and the saturated acids are corrected by subtracting the percentage of unsaponifiable matter, the content of fatty acids in the oil is 37.1% linoleic acid, 25.7% oleic acid, and 31.6% saturated acids.

No processing data for okraseed oil have been reported in the literature, and the data with respect to the fatty acid composition determined by various analytical methods are not consistent. In the present communication the results of a comprehensive investigation on the production, characteristics, composition, and stability of solvent-extracted and hydraulic-pressed okraseed oils and their hydrogenated products are reported.

Experimental

Material. The okraseed used in this investigation was of the Louisiana Green Velvet variety, grown at the Louisiana State Penitentiary, Angola, La., in 1947. The samples of cracked seed, hydraulic-pressed oil, and press cake were obtained during a mill scale processing test at the Southern Cotton Oil Company's oil mill, New Roads, La. The composition of the seed used for solvent extraction and hydraulic pressing is given in Table I.

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE I

Analysis of Okraseed Used for Solvent Extraction and Hydraulic Pressing

Constituent	Seed used for solvent extraction		Seed used for hydraulic pressing			
			Clean cracked seed		Cracked seed with trash	
	As rec'd basis, %	Moisture-free basis,%	As rec'd basis, %	Moisture-free basis,%	As rec'd basis, %	Moisture-free basis,%
Moisture	9.89	.....	11.09	.....	13.71	.....
Nitrogen	3.10	3.44	2.84	3.19	2.62	3.04
Protein (N×6.25)	19.38	21.50	17.75	19.90	16.38	18.98
Ash	4.74	5.26	4.41	4.96	4.28	4.96
Potassium	0.82	0.91	.....	.....	.....	.....
Phosphorus	0.79	0.87	.....	.....	.....	.....
Calcium	0.21	0.23	.....	.....	.....	.....
Crude fiber	21.02	23.32	26.55	29.86	28.22	32.70
Total sugar	4.03	4.47	.....	.....	.....	.....
Lipids	16.78	18.62	14.25	16.02	9.95	11.53

Solvent Extraction of the Oil. The okraseed was cracked between rolls set 0.030 in. apart and the unseparated kernels and hulls were flaked to a thickness of 0.010 in. Three batch extractors were charged with 130 pounds each of the flaked seed, having a moisture content of 11% and an oil content of 15.4%. The extractors were then filled with commercial hexane (b.p. 146°F.) and allowed to stand overnight. The extraction was carried out with 272.6 gallons of solvent at a temperature of 35 to 48°F. for 11¾ hours at a flow rate of 23.2 gal./hr. The yield of oil and meal was 52 lb. and 307 lb., respectively.

Extracted Meal. The compositions of the solvent-extracted meals from the three extractor cells were as follows: moisture 9.10, 9.30, and 9.40%; nitrogen 4.21, 5.10, and 3.82%; protein (nitrogen × 6.25) 26.31, 31.88, and 23.87%; crude fiber 22.62, 17.77, and 24.24%; and lipids 1.30, 1.60, and 1.89%, respectively. The hydraulic-pressed meal contained: moisture 10.49%, nitrogen 3.61%, protein (N × 6.25) 22.56%, crude fiber 28.21%, lipids 5.5% and ash 5.45%. The protein contents of both the extracted meals (22.5-31.8%) are comparable with that of undecorticated cottonseed meal (20.7%). The residual oil content of the solvent-extracted meal is appreciably lower than that of hydraulic-pressed meal.

Refined Oil. The refining tests on both solvent-extracted and hydraulic-pressed oils were conducted according to the conditions specified in the Official Methods of the American Oil Chemists' Society (7) for hydraulic cottonseed oil. The refining data for solvent-extracted and hydraulic-pressed oils are given in Table II. The oil produced by solvent extraction gave a low refining loss with 12° and 14° Bé. lyes. The refining loss was comparatively lower in the case of 14° Bé. lye although the difference in time of stirring in the cold bath (15 min. and 90 min.) had no appreciable effect on the refining loss. The hydraulic-pressed oil was dark in color and had a higher free fatty acid content than the solvent-extracted oil. It gave a higher refining loss than the solvent-extracted oil, with 14° and 18° Bé. lyes using slow and regular breaks. The lowest refining loss was obtained with 18° Bé. lye using the slow break method, *i.e.*, 45 minutes stirring in the cold bath. The refining tests, made with more concentrated lyes to obtain lighter colored oil, resulted in the production of very soft soapstock from the which the refined oil could not be separated quantitatively by decantation.

TABLE II

Refining Data on Solvent-Extracted and Hydraulic-Pressed Okraseed Oils

Lye		Time stirred in cold, min.	Time of stirring at 63-67°C., min.	Condition of soapstock	Refining loss	Lovibond color, (5¼ in. cell) 70 Y/R
°Bé.	% Max.					
Solvent-extracted oil, F.F.A. 0.4%						
12	80	15	12	Firm	2.6	3.0
12	80	90	12	Firm	2.7	3.0
14	80	15	12	Firm	3.9	3.0
14	80	90	12	Firm	3.7	3.0
Hydraulic-pressed oil, F.F.A. 3.5%						
14	100	15	12	Soft	17.3	7.7
14	100	45	20	Soft	17.0	7.7
18	100	15	12	Small grains, not very firm	15.3	6.4
18	100	45	20	Small grains, not very firm	14.3	6.4
20	.....[a]	15	12	Very soft[b]	.....	7.0
22	.....[a]	45	12	Very soft[b]	.....	7.0

[a] Calculated plus 0.5%.
[b] Refined oil could not be separated quantitatively by decantation.

Bleached Oil. The refined solvent-extracted okraseed oil had a greenish cast due to the presence of pheophytin A (7a), most of which was probably extracted from the hulls of the seed. The bleaching tests, carried out with acid activated earths under the conditions prescribed by the Official Methods of the American Oil Chemists' Society (7), gave light colored oils with only a slight greenish tinge. The refined hydraulic-pressed oil was very difficult to bleach to a light color. See Table III. The lowest color obtained after bleaching with various earths and carbons was 35 yellow/4.3 red Lovibond units.

TABLE III

Effect of Bleaching Solvent-Extracted and Hydraulic-Pressed Refined Okraseed Oils, With Various Adsorbents

Quantity and type of adsorbent used for bleaching	Color of bleached oils (5¼ in. cell), Yellow/Red
Solvent extracted oil	
"B. C. Clay,"[a] 4%	5/0.6
"B. C. Clay,"[a] 6%	5/0.4
"Activite" clay,[a] 4%	10/1.1
"Activite" clay,[a] 6%	5/0.8
Official Natural Earth, 6%	5/0.5
Official Natural Earth, 4%	10/0.5
Hydraulic pressed oil	
Official Activated Earth, 4%	35/4.9
Official Natural Earth, 6%	35/4.4
Activite clay, 4% + Nuchar, 0.4%[a]	35/4.3
Official Activated Earth, 4%	35/6.4

[a] These bleaching agents are named as part of the experimental conditions. Their use does not constitute a recommendation by the Department of Agriculture of these products over those of any other manufacturer of similar products.

Deodorization. The refined and bleached, solvent-extracted and hydraulic-pressed okraseed oils were steam deodorized at 220°C. and 1-mm. pressure for 2 hours, in a laboratory deodorizer described by Bailey and Feuge (8). Both the oils after deodorization were practically odorless. The deodorization of refined and bleached, hydraulic-pressed oil gave a light colored oil with a Lovibond color of 35 Yellow/2.2 red.

Characteristics of Crude and Refined Okraseed Oils. The characteristics of crude and refined, solvent-extracted and hydraulic-pressed oils were determined by the methods prescribed by the American Oil Chemists' Society (7), except for the unsaponifiable matter (9), thiocyanogen value (10), and hydroxyl value (11) which were determined by the methods in the references cited. The characteristics of okraseed oils are compared in Table IV with values previously reported in the literature.

TABLE IV

Physical and Chemical Characteristics of Solvent-Extracted and Hydraulic Pressed Okraseed Oils

Characteristics	Solvent-extracted		Hydraulic-pressed		Literature values[a]
	Crude	Refined	Crude	Refined	
Specific gravity, 25°/25°	0.9163	0.9153	0.9175	0.9165	0.9160-0.9187
Refractive index $n_D^{40°C.}$	1.4630	1.4634	1.4634	1.4632	1.4692[b]-1.4702
Free fatty acids, (as oleic),%	0.4	.....	3.5	.....	0.30-1.42
Saponification value	192.9	192.5	193.5	194.0	189-195.6
Iodine value, Wijs	91.7	92.3	94.1	94.7	91.1-111
Thiocyanogen value	60.6	60.3	61.7	61.8	59.2-64.5
Reichert-Meissl value	0.30	0.55	0.38	0.22	.09-.14
Polenske value	0.50	0.79	0.99	1.39	.23-.32
Hydroxyl value	4.8	4.3	4.3	4.4	.....
Unsaponifiable matter, %	1.40	1.30	2.2	2.1	.37-1.6

[a] See references 2, 5, and 6.
[b] Measured at 25° instead of 40°C.

Hydrogenation. The alkali-refined and bleached, solvent-extracted and hydraulic-pressed okraseed oils were hydrogenated under conditions of moderate selectivity and low iso-oleic acid production. The hydrogenation was carried out at 250°F. under 15 p.s.i. hydrogen pressure, with 0.1% dry-reduced, electrolytically-precipitated nickel catalyst (12) in the apparatus described by Bailey, Feuge, and Smith (13). The reduction in iodine value proceeded smoothly with marked reduction of color. In each case samples of hydrogenated oils were withdrawn at intervals of approximately 5 units reduction in iodine value. The hydrogenated products obtained were similar in appearance to hydrogenated cottonseed or peanut oils. Some of the characteristics of hydrogenated series of solvent-extracted (0-431) and hydraulic-pressed (0-452) okraseed oils are reported in Table V.

The relationship between refractive index and iodine value is very nearly the same for hydrogenated solvent-extracted and hydraulic-pressed oils.

Measurements of the consistencies of hydrogenated okraseed oils were made according to the micropenetration technique described by Feuge and Bailey (14) and with the results shown in Figures 1 and 2.

The interpolated micropenetrations of hydrogenated oils at a temperature of 22.5°C. are plotted against their iodine values (Figure 3). It will be noted from this figure that the variation of consistency of solvent-extracted and hydraulic-pressed okraseed oils is similar and for a given iodine value hydrogenated solvent-extracted oil is softer than hydraulic-pressed oil.

The stabilities of unhydrogenated and hydrogenated okraseed oils were determined by the active oxygen method (15) at 97.7°C. using a peroxide value of 100 milliequivalents per kilogram of fat as the end point. Stabilities of refined and bleached samples were 11.5 and 14 hours for hydraulic-pressed and solvent-extracted oils, respectively, compared to a cottonseed oil which had a stability of 11 hours under the same conditions. The stability of the hydrogenated products produced from the solvent-extracted oil was higher for a given iodine value and increased more rapidly by progressive hydrogenation than that of hydraulic-pressed oil as may be seen by reference to Table V.

Composition of Hydrogenated and Unhydrogenated Okraseed Oils. Analyses of solvent-extracted (0-431) and hydraulic-pressed (0-452) okraseed oils and their hydrogenated products in terms of their fatty acids on glyceride basis are given in Table VI. The fatty acid composition was determined by the following methods:

a) Calculation from iodine and thiocyanogen values using the equations of the American Oil Chemists' Society (7).

b) Calculation from spectrophotometric measurements using the method described in the Report of the Spectroscopy Committee of the American Oil Chemists' Society (16). The measurements were made on the oils before and after alkali isomerization for 25 minutes at 180°C. with an ethylene glycol-potassium hydroxide reagent protected with nitrogen.

c) Determination of saturated acids by the Pelikan and Von Mikusch modification (17) of Bertram oxidation method except that sintered glass filter sticks were used for filtering the magnesium soaps.

d) Determination of iso-oleic acid and saturated acids by modified Twitchell lead-salt separation method (7).

The major component fatty acids were found to be linoleic, oleic, and saturated acids; linolenic and arachidonic acid were present in negligible quantities. In Figures 4 and 5 the fatty acid composition of the original and hydrogenated oils calculated from their iodine and thiocyanogen values was used to show the change in composition with reduction in iodine values during hydrogenation because the results obtained by spectrophotometric analysis were not very consistent (Table VI). A linear relationship was observed when the reciprocal of keeping time (1/AOM) was plotted against the linoleic acid content for both series of hydrogenated oils (18).

TABLE V

Characteristics of Hydrogenated Okraseed Oils

Sample	Iodine value	AOM,[c] hours	1/AOM $\times 10^3$	Refractive index $n_D^{60°C.}$	Lovibond color Yellow/Red
0-431-0[a]	92.5	14	71.4	1.4632[b]	20/2.4
0-431-1	81.2	28	35.4	1.4547	10/0.9
0-431-2	75.7	38	26.3	1.4541	10/0.7
0-431-3	70.6	44	22.7	1.4534	5/0.9
0-431-4	65.8	61	16.3	1.4532	5/0.9
0-431-5	61.0	81	12.3	1.4529	5/0.9
0-431-6	56.2	149	6.7	1.4515	5/0.9
0-431-7	51.7	230	4.3	1.4509	5/0.8
0-431-8	46.5	245	4.0	1.4501	5/0.7
0-431-9	41.4	605	1.6	1.4499	5/0.6
0-452-0[a]	93.9	11½	86.9	1.4637[b]	35/4.9
0-452-1	83.2	14	74.0	1.4548	35/3.5
0-452-2	78.2	15¾	63.2	1.4540	35/3.2
0-452-3	73.6	18¾	54.0	1.4535	35/3.2
0-452-4	68.7	22	45.5	1.4530	35/2.9
0-452-5	64.3	27¼	37.0	1.4521	35/2.7
0-452-6	59.1	40	25.0	1.4518	35/2.7
0-452-7	54.9	140	7.1	1.4511	35/2.6
0-452-8	51.0	156	6.4	1.4510	35/2.4
0-452-9	46.4	282	4.0	1.4501	35/2.3

[a] Refined and bleached, unhydrogenated oils.
[b] Determined at 40°C.
[c] Keeping time in hours, by active oxygen method (15).

TABLE VI

Composition of Unhydrogenated and Hydrogenated Okraseed Oils

Sample	Iodine value	Thiocyanogen value	Fatty acid in oils, %								
			Linoleic		Oleic			Saturated			
					Total						
			A[a]	B[b]	A[a]	B[b]	Iso[d]	A[a]	B[b]	C[e]	D[d]
0-431-Crude	91.7	60.6	36.5	37.1	28.1	25.0	.....	30.8	32.7	.....	.....
0-431-0[e]	92.5	60.4	37.9	34.7	26.4	31.3	.....	31.3	28.9	30.9	.....
0-431-1	81.2	58.6	26.6	28.2	36.7	33.2	3.2	32.3	34.1	31.3	31.9
0-431-2	75.7	58.6	20.5	21.0	42.7	41.5	.....	32.2	32.9	31.5	.....
0-431-3	70.6	57.5	15.0	15.8	48.0	46.5	6.1	32.5	33.2	31.8	32.2
0-431-4	65.8	56.7	10.3	9.6	52.1	53.8	.....	33.0	33.7	33.5	.....
0-431-5	61.0	55.6	6.0	7.0	55.6	53.6	8.2	34.0	34.9	34.4	33.7
0-431-6	56.2	54.1	2.2	2.9	58.0	56.6	.....	35.4	36.0	36.5	.....
0.431.7	51.7	51.0	0.5	0.7	56.4	55.9	7.9	38.6	38.9	.....	36.3
0-431-8	46.5	46.1	0.1	0.2	51.4	51.3	.....	44.0	44.1	.....	.....
0-431-9	41.4	41.5	0.0	0.1	46.8	45.8	.....	48.8	49.7	.....	.....
0-452-Crude	94.1	61.7	38.2	42.0	27.7	17.7	.....	29.7	35.1	.....	.....
0-452-0[e]	93.9	60.4	39.6	41.3	24.6	19.0	.....	31.4	34.3	30.8	.....
0-452-1	83.2	59.3	28.2	29.0	35.7	33.5	2.5	31.7	32.7	30.9	32.4
0-452-2	78.2	59.2	22.4	24.6	41.9	37.0	.....	30.9	33.2	30.0	.....
0-452-3	73.6	58.4	17.7	17.7	46.1	45.8	6.6	31.8	31.9	31.0	31.7
0-452-4	68.7	57.5	12.9	13.5	50.8	48.9	.....	31.8	33.0	31.0	.....
0-452-5	64.3	56.6	8.8	8.5	54.0	54.3	8.0	32.9	32.8	32.9	33.0
0-452-6	59.1	55.5	3.9	3.7	57.7	58.1	.....	33.8	33.7	34.2	.....
0-452-7	54.9	53.4	1.4	1.0	58.1	58.8	10.9	36.1	35.7	.....	36.0
0-452-8	51.0	50.3	0.5	0.3	55.6	55.8	.....	39.4	39.4	.....	.....
0-452-9	46.4	45.9	0.2	0.1	51.1	51.4	.....	44.3	44.1	.....	.....

[a] Iodine-thiocyanogen method, saturated acids by difference.
[b] Spectrophotometric method; oleic acid calculated from iodine value; saturated acids by difference.
[c] Modified Bertram oxidation method.
[d] Modified Twitchell lead-salt alcohol method. (A.O.C.S.)
[e] Refined and bleached oil before hydrogenation.

Linoleic Acid. In the early experiments the linoleic acid content of unhydrogenated and slightly hydrogenated okraseed oils determined by spectrophotometric measurements was appreciably lower than that calculated from iodine and thiocyanogen values. However, it was found that isomerization with vigorous shaking gave fairly good checks for the duplicates and agreement with calculated composition from iodine and thiocyanogen values. The low values for linoleic acid which are sometimes obtained by this method are believed to result from incomplete saponification and isomerization of the oil.

The values for linoleic acid obtained by the spectrophotometric method reported in Table VI were calculated from values for the extinction coefficients after alkali isomerization for linoleic acid prepared by the bromination-debromination procedure. Use of the extinction coefficients proposed by Swain *et al.* (19) and obtained by alkali isomerization of linoleic acid prepared by solvent crystallization and chromatography would decrease the calculated values for linoleic acid by the spectrophotometric method by a factor of *ca.* 5%. Thus, for example, the value of 42.0% linoleic acid reported for 0-452-crude would be reduced to 39.9% linoleic acid.

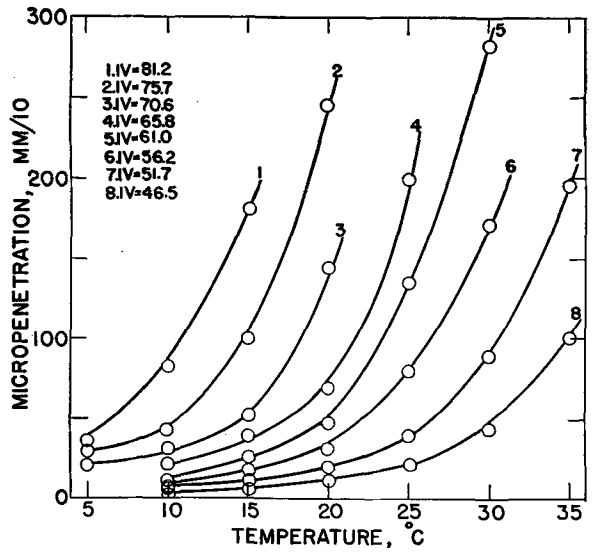

FIG. 1. Micropenetration data for solvent-extracted okraseed oil hydrogenated to varying degrees of hardness.

Oleic Acid. In the hydrogenation of both solvent-extracted and hydraulic-pressed okraseed oils, the oleic acid content increases to a maximum and thereafter declines (Figures 4 and 5) as in the case of other oils whose unsaturated acids consist substantially of oleic and linoleic acids. The oleic acid contents of unhydrogenated and slightly hydrogenated oils calculated from spectrophotometric measurements are lower than those calculated from iodine and thiocyanogen values; particularly for unhydrogenated hydraulic pressed oils.

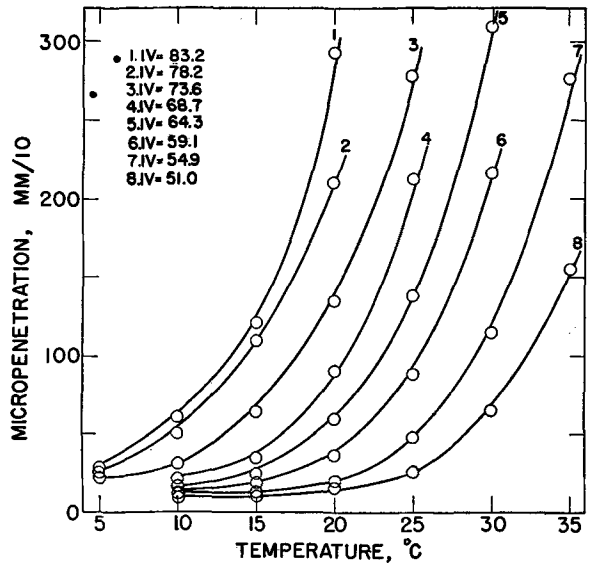

FIG. 2. Micropenetration data for hydraulic-pressed okraseed oil hydrogenated to varying degrees of hardness.

Saturated Acids. The contents of saturated acids determined by different methods were in fairly good agreement. However the values obtained for most of

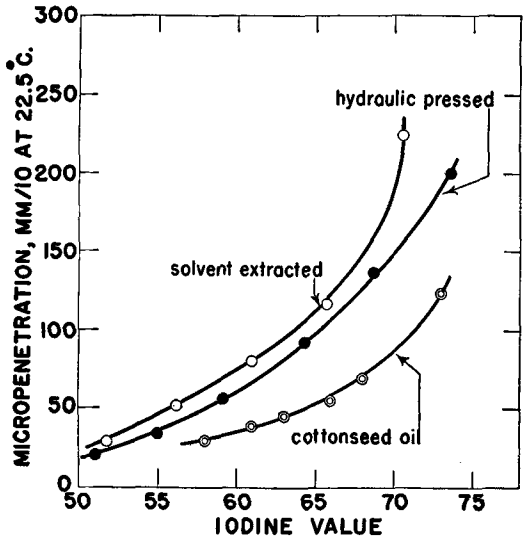

FIG. 3. Variation of consistency of solvent-extracted and hydraulic-pressed okraseed oils at 22.5°C. by the standard method.

the unhydrogenated and slightly hydrogenated oils by spectrophotometric methods were higher than those obtained by chemical methods.

Summary

The characteristics and properties of okraseed oils produced by solvent extraction and hydraulic pressing have been investigated. The refining and bleaching of solvent-extracted oil gave a very light colored product (10 yellow and 1.0 red, bleached with 4% acid-activated clay), however, the lowest color obtained on bleaching the refined hydraulic-pressed oil with several adsorbent earths was 35 yellow/4.3 red Lovibond units. Deodorization of this refined and bleached oil reduced the color to 35 yellow/2.2 red. The hydrogenation of both solvent-extracted and hydraulic-pressed okraseed oils gave products similar to hydrogenated cottonseed oils. The characteristic properties of crude, refined, and hydrogenated okraseed oils were determined.

Micropenetration values for the hydrogenated oils showed that solvent-extracted oil was softer than hydraulic-pressed oil for a given iodine value. The keeping time by the active oxygen method for both solvent-extracted and hydraulic-pressed okraseed oils (14 and 11.5 hrs.) was comparable to that of high grade cottonseed oil (*ca.* 11 hrs.). The fatty acid compositions of the solvent-extracted and hydraulic-pressed okraseed oils calculated from the iodine and thiocyanogen values were respectively, 36.5 and 38.2% linoleic acid, 28.1 and 27.7% oleic acid, and 30.8 and 29.7% saturated acids. Discrepancies between these results and those obtained in early spectrophotometric measurements were observed, however by repeated isomerizations fairly good agreement was obtained.

The results reported here indicate that hydraulic-pressed oil is somewhat inferior in color and stability to solvent-extracted oil. Both oils however can be used as substitutes for the edible oils now in commercial use, particularly for cottonseed oil.

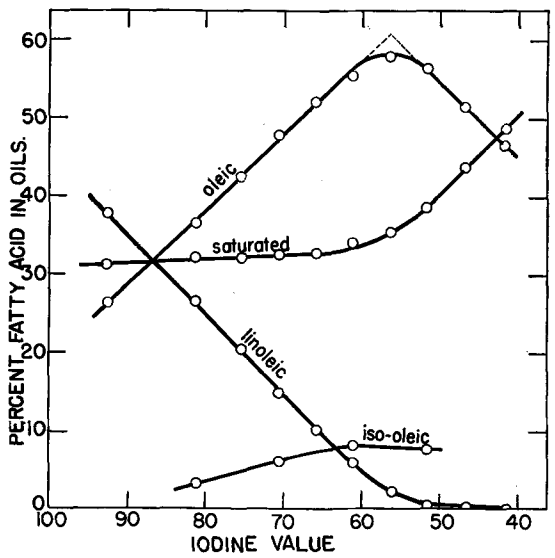

FIG. 4. Composition of solvent-extracted okraseed oil hydrogenated to varying degrees of hardness.

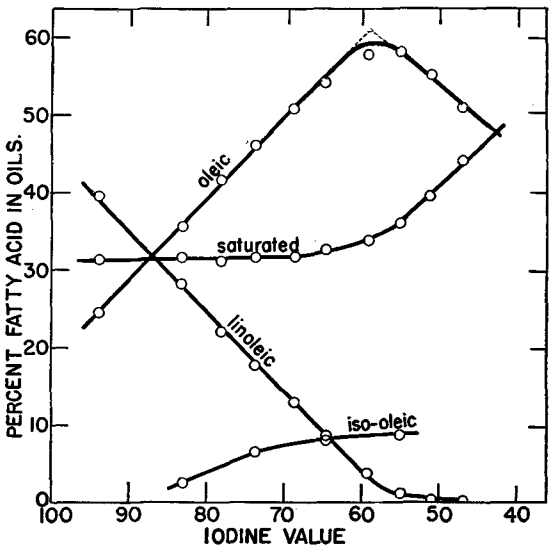

FIG. 5. Composition of hydraulic-pressed okraseed oil hydrogenated to varying degrees of hardness.

Acknowledgment

The authors wish to express their appreciation to L. J. Moliason for his assistance in solvent-extraction of okraseed; to A. F. Cucullu, V. O. Cirino, E. R. McCall, and C. Lesslie for the analysis of okraseed and extracted meal; Josephine R. Loeb for thiocyanogen values, and R. T. O'Connor and E. T. Field for the spectrophotometric absorption data reported in this paper.

REFERENCES

1. Edwards, W. R., Jr., and Miller, J. C., The Chemurgic Digest, *6* (No. 2), 29, 31-33 (1947).
2. Markley, K. S., and Dollear, F. G., "Okraseed as a Potential Oilseed Crop," Chemurgic Papers 1947 Series, No. 3, Paper No. 576, National Farm Chemurgic Council (1947).
3. Kilgore, B. W., "Chemical Analyses of Some Native North Carolina Grasses, Forage Plants, Grains, Seeds, and By-products." North Carolina Agr. Experiment Station, Bulletin No. 90b (1893).
4. Halverson, J. O., and Naiman, B., J. Oil & Fat Ind., *3*, 386-387 (1926).
5. Clopton, J. R., Roberts, A., and Jeskey, H. A., J. Am. Oil Chem. Soc., *25*, 401-404 (1948).
6. Jamieson, G. S., and Baughman, W. F., J. Am. Chem. Soc., *42*, 166-170 (1920).
7. American Oil Chemists' Society Official and Tentative Methods, 2nd ed., ed. by V. C. Mehlenbacher, Chicago, 1946.
7a. O'Connor, R. T., Field, E. T., Jefferson, M. E., and Dollear, F. G., J. Am. Oil Chem. Soc., *26*, 710-718 (1949).
8. Bailey, A. E., and Feuge, R. O., Ind. Eng. Chem., Anal. Ed., *15*, 280-281 (1943).
9. Cocks, L. V., "Report of the Subcommittee on Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps," Analyst, *58*, 203-211 (1933).

10. Lambou, M. G., and Dollear, F. G., Oil & Soap, *22*, 226-232 (1945).
11. West, E. S., Hoagland, C. L., and Curtis, G. H., J. Biol. Chem., *104*, 627-634 (1934).
12. Bailey, A. E., "Industrial Oil and Fat Products," Interscience Publishers, New York, 1945, pp. 591-593.
13. Bailey, A. E., Feuge, R. O., and Smith, B. A., Oil & Soap, *19*, 169-176 (1942).
14. Feuge, R. O., and Bailey, A. E., Oil & Soap, *21*, 78-84 (1944).
15. "Report of the Committee on Analysis of Commercial Fats and Oils," Oil & Soap, *22*, 101-107 (1945).
16. "Report of the Spectroscopy Committee," J. Am. Oil Chem. Soc., *16*, 399-404 (1949).
17. Pelikan, K. A., and Von Mikusch, J. D., Oil & Soap *15*, 149-150 (1938).
18. Fisher, G. S., Bickford, W. G., and Dollear, F. G., J. Am. Oil Chem. Soc., *24*, 379-382 (1947).
19. Swain, M. S., Brice, B. A., Nichols, P. L., Jr., and Riemenschneider, R. W., "Revised Constants and Calculations for the Spectrophotometric Determination of Polyunsaturated Fatty Acids." Presented at the 22nd Fall Meeting of the American Oil Chemists' Society, New York, Nov. 15-17, 1948.

[Received December 6, 1949]

Effect of Fractionation and Treatment on the Acute Oral Toxicity and on the Gossypol and Gossypurpurin Content of Cottonseed Pigment Glands[1,2]

EDWARD EAGLE, Research Laboratories, Swift and Company, Chicago, Illinois,[3] and CATHERINE M. HALL, LEAH E. CASTILLON, and CHARLOTTE BOATNER MILLER, Southern Regional Research Laboratory,[4] New Orleans, Louisiana

SINCE the original report (1) on the acute oral toxicity of gossypol and cottonseed pigment glands for rats, mice, rabbits, and guinea pigs, a total of 21 fractionated and variously treated cottonseed pigment gland preparations have been tested for their acute oral toxicity (LD_{50} value) and for their content of extractable gossypol and gossypurpurin; and the acute oral toxicity of six different samples of pure gossypol has been determined.

Experimental

The first three samples of untreated cottonseed pigment glands (1*b*, 2*b*, and 3*a*) used in this study (Table 1) were prepared by the gland flotation process (2, 3) from a single lot of prime cottonseed (1945 crop) which was stored in a silo for about 6 months before processing. Sample 1*b* was a composite of pigment glands prepared from seed processed between March 8 and April 16, 1946; sample 2*b* was obtained from seed processed between May 16 and May 25, 1946; sample 3*a* was made from seed processed between November 18 and December 11, 1946. The separated glands were stored at 7°C. in sealed containers prior to administration. Sample 4*a* of untreated pigment glands was isolated from an entirely different lot of prime cottonseed, 1946 crop, which had been stored for about six months prior to processing in July and August, 1947. This seed was defatted with commercial hexane (boiling point range of 146-158° F.) prior to removal of the pigment glands.

The various fractions (1*a* to 1*h*) were prepared as follows: 100 grams of pigment glands (sample 1*b*) were blended with 400 ml. acetone for 5 min. in a Waring Blendor, the mixture was centrifuged, the supernatant was decanted and the residue was repeatedly washed with small volumes of acetone until the total volume of acetone extract equaled 400 ml. The residue, consisting mainly of gland walls and a small amount of adhering gossypol, was dried in a vacuum desiccator at room temperature. This residue was called the acetone-insoluble fraction (sample 1*h*). To the above acetone extract 400 ml. distilled water was added, the mixture was stirred and centrifuged, and the supernatant was filtered. Most of the acetone in the filtrate was evaporated at room temperature and under vacuum. The water was removed from the remaining portion of this supernatant by lyophilizing to give sample 1*a*. The viscous, oily, reddish-yellow material, resulting from the addition of water to the original acetone extract and recovered by centrifugation, was freed of acetone and water in a vacuum desiccator at room temperature. Then the residue was washed with light petroleum naphtha (boiling point range of 60°-110°F.) until all of the reddish-brown viscous component was removed. The residue, solid and brown in color, was dried overnight in a vacuum desiccator and was sample 1*e*. The light petroleum naphtha wash solutions were combined and evaporated under vacuum at room temperature to give the dark brown, viscous product (sample 1*g*).

A total of 400 grams of pigment glands from the same lot (sample 1*b*) was mixed with 1,600 ml. distilled water at a temperature of 3.5°C. and the mixture was centrifuged at 1,800 r.p.m. at 2°C. The precipitate was composed principally of gland walls and some gossypol (sample 1*d*). The water from the cloudy supernatant was removed at low temperature under vacuum and the dried product was sample 1*c*. A 221-gram portion of the lyophilized product (sample 1*c*) was suspended in 400 ml. water and centrifuged at high speed (10,000 r.p.m.) for 2½ hours at 2°C. The supernatant was decanted and lyophilized (sample 1*f*).

A diagram of the procedures used in the fractionation of the pigment glands and of the products obtained is given in Figure 1.

To determine the effect of heat on the toxicity of cottonseed pigment glands, samples were placed in covered aluminum pans and heated at the temperatures indicated (samples 2*c* and 3*c*). Sample 2*a* was made by grinding some of sample 2*c* in a Wiley Mill through a ½-mm. screen. To ascertain the effect of heat in the presence of water, the pigment gland samples were thoroughly wetted with distilled water and

[1] Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946.

[2] Presented at fall meeting, American Oil Chemists' Society, Chicago, Oct. 31, Nov. 1-2, 1949.

[3] This work was conducted with Swift and Company under a memorandum of understanding with the Bureau of Agricultural and Industrial Chemistry. The preparation of the samples of gossypol and cottonseed pigment glands and the analyses for gossypol and gossypurpurin content were carried out in the Southern Regional Research Laboratory; all of the animal experiments were performed in the Research Laboratories of Swift and Company.

[4] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.