value of 100 introduces only a negligible error. Reference to Figures 3, 4, and 5 and corresponding data given in the tables clearly illustrate this point.

Summary

Thirteen compounds have been intercompared with respect to their antioxidant activity in concentrations of 0.01, 0.05, and 0.10% in edible cottonseed oil, the same oil hydrogenated to shortening consistency, and in lard which is essentially free of naturally occurring antioxidants. None of the compounds exhibited significant antioxidant activity in the cottonseed oils, when used in a concentration of 0.01%, but they were effective in stabilizing lard under these conditions. Propyl gallate was the most effective of the compounds tested for the vegetable fats.

Examination of the data for lard indicates that the comparison of antioxidants in this and other substrates essentially devoid of natural inhibitors may yield more realistic results when compared at a peroxide level of 100 milliequivalents per kilogram of substrate.

REFERENCES

1. Bailey, A. E., "Industrial Oil and Fat Products," Interscience Publishers Inc., New York, 1945, pp. 591-593.
2. Bailey, A. E., and Feuge, R. O., Ind. Eng. Chem., Anal. Ed., *15*, 280-281 (1943).
3. Bailey, A. E., Feuge,, R. O., and Smith, B. A., Oil and Soap, *19*, 169-176 (1942).
4. Budowski, Pierre, J. Am. Oil Chem. Soc., *27*, 264-267 (1950).
5. Committee on Analysis of Commercial Fats and Oils, Ind. Eng. Chem., Anal. Ed., *17*, 336-340 (1945).
6. Fisher, G. S., Kyame, Lillian, and Bickford, W. G., J. Am. Oil Chem. Soc., *24*, 340-343 (1947).
7. Fore, Sarah P., Moore, R. N., and Bickford, W. G., J. Am. Oil Chem. Soc., *28*, 73-74 (1951).
8. King, A. E., Roschen, H. L., and Irwin, W. H., Oil and Soap, *10*, 105-109 (1933).
9. Mattil, K. F., Filer, L. J., Jr., and Longenecker, H. E., Oil and Soap, *21*, 160-161 (1944).
10. Swift, C. E., Mann, G. E., and Fisher, G. S., Oil and Soap, *21*, 317-320 (1944).

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Buckwheat Leaf Meal Fat. II. Composition of the Fatty Acids

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A PREVIOUS paper (5) describes the preparation of buckwheat leaf meal fat, reports its physical and chemical characteristics, and gives an account of the identification of the constituents in the unsaponifiable matter and the water-soluble fraction of the saponified fat. This manuscript describes the characterization of the chief constituents in the water-insoluble fraction of the saponified fat.

Purification of the Water-Insoluble Fatty Acids. According to Official Methods (1), the value for insoluble acids includes the unsaponifiable matter. The quantity of unsaponifiable matter is inconsequential in most fats and oils that contain these constituents in low concentration, but since buckwheat fat contains a high percentage (14.9%) of this material, the value of 64.9% previously reported (5) for the insoluble acids did not include the unsaponifiable matter.

The insoluble acids (free of unsaponifiable matter) contain a quantity of material not of fatty acid character. Exhaustive extraction of a 221.8-g. sample of the insoluble acids with petroleum ether (boiling range 63-70°C.) gave 158.6 g. (71.5%) of soluble material and 63.2 g. (28.5%) of insoluble material. On the basis of the original fat the petroleum-ether soluble material (fatty acids) was 46.6%, and the insoluble material was 18.5%.

To free the purified fatty acids of a small amount of coloring matter, an 89.4-g. sample of the soluble material was dissolved in petroleum ether and treated with 40.0 g. of Norit. To determine whether appreciable quantities of individual acids were preferentially adsorbed by Norit, untreated and Norit-treated samples were submitted to spectrophotometric analyses (2, 3) for polyunsaturated acids and to various chemical analyses for analytical characteristics. Table I shows the results of these analyses.

TABLE I

Spectrophotometric and Analytical Characteristics of Purified Mixed Fatty Acids of Buckwheat Leaf Meal Fat

	Pigment-contaminated fatty acids	Pigment-free fatty acids
Peroxide value (milliequivalents/kilo)	24.2	25.9
Iodine value (Wijs)	154.8	163.3
Neutralization equivalent	296.0	285.7
Hydroxyl value (%)		0.1
Saturated acid content (%)[a]	21.8	22.0
Saturated acids by difference (%)	17.2	19.7
Neutralization equivalent, saturated acids from Bertram analysis[b]	295.0	282.2
Iodine value, saturated acids from Bertram analysis		0.4
Oleic acid, calculated from iodine value and spectrophotometric analyses (%)	28.2	18.7
Linoleic acid (%)	18.2	20.9
Linolenic acid (%)	32.5	37.5
Preformed conjugated dienoic acids (%)	3.5	3.0
Ratio linolenic acid/linoleic acid	1.797	1.794

[a] Bertram method as modified by G. S. Jamieson, "Vegetable Oils and Fats," A.C.S. Monograph No. 58, 2nd Edition, 414-415 (1943).
[b] A single determination of neutralization equivalent was made on the saturated acids obtained from the Bertram analysis.

Both samples showed some spectroscopic evidence of oxidation by their appreciable diene absorption before isomerism and their tetraenoic absorption on heating in the absence of alkali (10). They did not appear to have reached a high state of oxidation however, and the differences in the degrees of oxidation of the samples were minor. The peroxide values of the samples substantiated the spectrophotometric findings. The linolenic/linoleic acid ratio alone indicates that Norit exerts little fractionating effect on the fatty acids. Calculations made from spectrophotometric analyses and iodine values suggest however that oleic acid may be preferentially absorbed by Norit.

Purification of the fatty acids may remove some substance which offers interference in the linolenic acid determination. Any analytical error in this determination would be multiplied in the calculation of oleic acid. In view of the uncertainty concerning

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the preferential adsorption of oleic acid, the values for the unsaturated acids contained on the pigment-contaminated fraction were considered more representative, and these values were used to calculate the unsaturated acid composition of the original fat. Also polyunsaturated acids have been calculated as percentages of linoleic and linolenic acid, the commonly occurring nonconjugated diene and triene unsaturated acids, but it should be emphasized that acids of any chain length having equivalent degrees of unsaturation would yield spectra similar to these.

Examination of the Mixed Fatty Acids. An attempt was made to characterize the buckwheat fatty acids by converting the purified acids to methyl esters and submitting them to distillation at 2 mm. Hg. pressure through a 13-mm. by 36-in. precision-spaced wire-packed (8) adiabatic fractionating column. Each of the 22 fractions separated was examined by chemical analysis for saponification equivalent, for unsaturation by hydrogenation, and spectrophotometrically for polyunsaturated acids.

Although the samples were protected by storage under a nitrogen atmosphere, the spectrophotometric analysis for polyunsaturated acids showed that these fractions had autoxidized to degrees ranging from mild to advanced so that no completely satisfactory analysis was possible. Fraction 14, the largest, 10.58 g. from a pot charge of 119.4 g., showed the least autoxidation; and fraction 21, 4.91 g., showed the most autoxidation. Only data for these two fractions (which are representative) are shown in Table II.

TABLE II

Composition of Representative Methyl Ester Fractions of Mixed Fatty Acids

Fraction	Linoleic acid, %	Linolenic acid, %	Preformed diene as conjugated linoleic acid, %	Saponification equivalent	Iodine[a] value
14	24.1	52.1	0.81	294.2	207.6
21	10.6	53.7	14.0	286.5	185.3

[a] Calculated from the degree of unsaturation found by hydrogenation.

Separation of Saturated from Unsaturated Acids. A 200-g. sample of the purified pigment-free fatty acids was separated into saturated and unsaturated acid fractions by crystallization from 1 liter of acetone at —20°C. The saturated acids were further freed of unsaturated acids by recrystallization from acetone (1 to 10) at —20°C. An iodine value of 3.09 indicated relative freedom from unsaturated acids. A 24-g. sample of the saturated acids was converted to methyl esters by dissolving it in 41 ml. of absolute methanol, adding a solution containing 0.48 g. of concentrated sulfuric acid in 7 ml. of methanol, and refluxing for 5 hours. After cooling, 200 ml. of water were added, and the esters were extracted with ether and washed in the usual manner. The dried methyl esters had an iodine value of 2.90 (calculated from hydrogen uptake measurements). Assuming the only unsaturated ester present to be oleic acid, the amount of this ester was 3.23%.

Fractionation of Saturated Acid Methyl Esters. An 11.79-g. sample of the saturated methyl esters was distilled through a 7-mm. by 18-inch miniature, precision-spaced wire-packed adiabatic fractionating column.[2] Table III gives the composition of the fractions obtained.

TABLE III

Composition of the Saturated Methyl Esters[a] of Buckwheat Leaf Meal Fat

Fraction No.	Fraction wt., g.	n_D^{50}	Ester C_{16} acid, %	Methyl oleate,[b] %	Ester C_{18} acid,[c] %	Ester C_{20} acid,[c] %	Ester C_{26} acid, %	Ester C_{28} acid, %
1	0.180	1.4293	100					
2	0.215	1.4294	100					
3	0.225	1.4294	100					
4	0.960	1.4294	100					
5	1.025	1.4293	100					
6	1.305	1.4293	100					
7	0.553	1.4293	100					
8	0.860	1.4293	100					
9	0.640	1.4294	100					
10	0.390	1.4294	100					
11	0.390	1.4294	100					
12	0.245	1.4294	100					
13	0.562	1.4294	100					
14	0.665	1.4294	100					
15	0.580	1.4297	100					
16	0.155	1.4339		26.6	73.4			
17	0.195	1.4355		32.1	67.9			
18	0.185	1.4340		20.5	79.5			
19	0.275	1.4339		9.9	66.2	24.0		
20	0.307	1.4339		13.2	47.9	38.8		
21[d]	1.075	1.4384					100[e]	
22[f]	0.420	1.4349[g]						100[h]
23[i]	0.140	1.4369						
24[j]	0.245							
Total	11.792		74.6	1.78	6.13	1.57	9.12	3.56
Total, corr.[k]			78.5	......	6.45	1.65	9.60	3.75

[a] Distilled at 0.90-0.40 mm. Hg. pressure.
[b] Calculated from hydrogen No., assuming methyl oleate to be the only unsaturated ester present.
[c] Calculated from hydrogen No. and saponification equivalent.
[d] A fraction obtained by sudden introduction of nitrogen into still pot.
[e] X-ray data show presence of traces of C_{24} and C_{28} acid esters.
[f] Column drainage.
[g] Reading at 80°C. Fraction was solid at 50°C.
[h] X-ray data show presence of a trace of C_{30} or higher acid esters.
[i] Fraction-cutter washings.
[j] Still-pot residue.
[k] For fractions 23 and 24 and for methyl oleate.

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[2] Distillation of a known mixture of methyl myristate, methyl palmitate, and methyl stearate showed that the column was highly efficient in their separation. In the preliminary tests flooding could not be controlled between 0.4 and 10 mm. Hg. pressure using a capillary nitrogen bleeder. It was effectively controlled however below 2.5 mm. Hg. pressure with the use of a magnetic stirrer. Small fraction cuts were taken, and their indices of refraction were read immediately, to follow methyl ester separation. Little attention was given to product or pot temperatures although these were measured and recorded by potentiometer during the fractionation. These temperatures fluctuated considerably owing to pressure drop in the column. By a manually operated needle valve, the reflux to product take-off ratio was maintained at not less than 10 to 1.

TABLE IV

Analyses of Methyl Esters of Saturated Acids Prepared from Buckwheat Leaf Meal Fat

Fraction No.	Iodine value[a]	Saponification equivalent[b]	X-ray powder pattern identification of methyl esters of saturated acids with carbon content of							
			C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}	C_{30} or higher
16	22.8	301								
17	27.5	301								
18	17.6	297								
19	8.5	306	......[c]	Major	Minor	......	......	......	......	......
20	11.3	311	......	Major	Minor	......	......	......	......	......
21	∞	......[d]	......	......	......	......	Trace?	Major	Trace?	......
22	∞	......[d]	......	......	......	......	......	......	Major	Trace?

[a] Calculated from the degree of unsaturation found by hydrogenation.

[b] A modified method for micro samples by C. L. Ogg and C. O. Willits of this laboratory. (Manuscript in preparation.)

[c] ...... means none detected.

[d] Values not determined because the high molecular weight acids were solvent insoluble.

The indices of refraction (6) show that the first 15 fractions are pure methyl palmitate. X-ray powder patterns prepared by L. P. Witnauer (11) support this conclusion. The remaining fractions, 16 to 22, were analyzed for saponification equivalents and for unsaturation by hydrogenation values; also fractions 19 to 22 were submitted to x-ray examination. Table IV shows the results of these analyses. This information was used to calculate the composition of fractions 16 to 22 inclusive. Only 1.78% of the 3.23% methyl oleate present in the original methyl ester mixture was accounted for in the distilled fractions. The difference was probably represented by decomposition material retained in the still residue.

Table V presents a summary of the organic acid composition of the buckwheat leaf meal fat calculated from the data obtained in this investigation.

TABLE V

Organic Acid Composition of Buckwheat Leaf Meal Fat

Acid	In saturated insoluble acids,%	In mixed insoluble acids,%	In the original fat,%
Saturated			
Formic[a]			0.27
Acetic[a]			Trace
Lactic[a]			0.87
Palmitic	78.3	17.1	7.97
Stearic	6.47	1.41	0.66
Arachidic	1.66	0.36	0.17
Behenic	None	None	None
Lignoceric	Trace	Trace	Trace
Hexacosanoic	9.75	2.13	0.99
Octacosanoic	3.87	0.83	0.39
Tricontanoic	Trace	Trace	Trace
Unsaturated			
Oleic		28.2	13.1
Linoleic		18.2	8.48
Linolenic		32.5	15.1
Preformed			
Conjugated			
Dienoic		3.5	1.63
Total	99.99	104.23	49.63

[a] Previously reported (5).

Petroleum-Ether Insoluble Material of the Water-Insoluble Fraction. The large quantity of petroleum-ether insoluble material found in the water-insoluble fraction (28.5% of the fraction) of the saponified fat indicates that the original fat probably contained, in addition to red pigment (5), large quantities of chlorophyll degradation products as well as the chlorophyll (2.8%) found by analysis (5).

A spectrophotometric examination of this material gave an absorption spectrum of the general shape characteristic of chlorophyll degradation products with maxima most pronounced at about 658, 498, and 410 mμ. and less pronounced at about 598 and 558. No individual compound was identified, but there was spectral similarity to that of phylloerythrin (4).

The solubility behavior of this material resembled that of the porphyrins. The best solvent among those tried was pyridine, in which it was soluble to the extent of about 20 g. in 100 ml. of boiling solution. Formic and glacial acetic acids were also good solvents. It was moderately soluble in absolute ethanol, acetone, strong hydrochloric acid, and ammonium hydroxide solutions. It was only slightly soluble in ether, ethyl acetate, and benzene. In water and petroleum-ether it was insoluble. Where soluble, except in hydrochloric acid solution, it imparted an olive-green color to the solvent. These solutions fluoresced red when exposed to ultra-violet light. The hydrochloric acid solution was emerald green and did not fluoresce. Dilute pyridine solutions were olive green but strong ones were red.

The ash content of this petroleum-ether material was relatively low, 0.58%, and the metals present were chiefly iron and magnesium.

An attempt was made to separate and identify the porphyrins by partition chromatography according to the technique of Rimington (9). Results showed the presence of only monocarboxylic acid type compounds such as phylloerythrin.

R_F value for phylloerythrin, 0.9; found, 0.89.

Values for carbon and hydrogen were in fairly close agreement with those calculated for phylloerythrin.

Anal. Calc'd for $C_{33}H_{34}N_4O_3 \cdot H_2O$: Carbon, 71.72%; hydrogen, 6.56%. Found: Carbon, 69.28%; hydrogen, 6.82%.[3] Moisture: Calc'd, 3.26%; found, 3.37%.

Satisfactory values for nitrogen analysis could not be obtained either by Kjeldahl or by Dumas method.

Summary

The composition of fatty acids of the saponified fat of buckwheat leaf meal was investigated. The mixed fatty acids amounted to 49.6% of the composition of the fat. The chief organic acids found were: linolenic, 15.1%; oleic, 13.1%; linoleic, 8.5%; and palmitic, 8.0%. Less than 1% each of such acids as formic, lactic, stearic, arachidic, hexacosanoic, and octacosanoic, and only traces of acetic, lignoceric, and tricontanoic (or higher) acids were found. The so-called "insoluble acids" of the saponified fat, less the unsaponifiable matter, contained 28.5% (18.5% based on the original fat) of material identified as red pigment and probably a mixture of chlorophyll degradation compounds, chiefly phylloerythrin.

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[3] Averages of two determinations. Corrected for ash.

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REFERENCES

1. A.O.A.C., "Official and Tentative Methods of Analysis," Ch. 31, Oils, Fats, and Waxes, 6th edition (1945).
2. Brice, B. A., and Swain, M. L., J. Optical Soc. Am., *35*, 532-544 (1945).
3. Brice, B. A., Swain, M. L., Schaeffer, B. B., and Ault, W. C., Oil and Soap, *22*, 219-224 (1945).
4. Fischer, H., and Gibian, H., Ann. 550, 208-251 (1942).
5. Krewson, C. F., and Couch, J. F., J. Am. Oil Chem. Soc., *28*, 382-385 (1951).
6. Krewson, C. F., J. Am. Chem. Soc., *73*, 1365 (1951).
7. Ogg, C. L., and Cooper, F. J., Anal. Chem., *21*, 1400-1402 (1949).
8. Podbielniak, W. J., Ind. Eng. Chem., Anal. Ed., *13*, 639-645 (1941).
9. Rimington, C., Biochemical Society Symposia No. 3. Partition Chromatography. Cambridge Univ. Press, 72-73 (1950).
10. Swain, M. L., and Brice, B. A., J. Am. Oil Chem. Soc., *26*, 272-277 (1949).
11. Witnauer, L. P., Nichols, P. L., and Senti, F. R., J. Am. Oil Chem. Soc., *26*, 653-655 (1949).

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Polymerization of Styrene in the Presence of Fatty Acid Methyl Esters[1]

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IN the preceding paper on the copolymerization of vegetable oils and styrene we were unable to draw any conclusions on whether the oils copolymerize with styrene or whether the reaction products are mixtures of oils and polystyrene (4). Satisfactory separation procedures for polystyrene and triglycerides were not available, and we studied therefore the copolymerization of styrene with methyl esters of fatty acids which can be separated from polystyrene by fractionation with methanol. This gave also an opportunity to investigate the effect of structural variations and of different functional groups of methyl esters on the polymerization with styrene. The object of this paper is to present our findings.

Experimental

Materials. Purified oleic acid was prepared from the Fischer U.S.P. product, using the crystallization procedure of Swern *et al.* (22). The material had an iodine value of 87.4 and contained less than 1.5% linoleic acid according to spectrophotometric analysis. The acid was used to prepare the methyl ester.

Elaidic acid was obtained from the purified oleic acid by isomerization with nitrous acid as described by Kass and Burr (10). The product had a melting point of 44°C. and an iodine value of 87.9. The acid was esterified with methanol.

Methyl ricinoleate was obtained from castor oil methyl esters by fractionation through a Vigreux column at 1 mm. Hg. (11).

Methyl 12-hydroxystearate was prepared from ricinoleate ester by hydrogenation, using Raney Nickel catalyst at 40 pounds' hydrogen pressure at 85-90°C. (6).

Methyl 12-ketostearate was obtained from the hydroxy analogue by oxidation with chromic acid in acetic acid solution (6).

Methyl 9,12-linoleate was prepared from tetrabromostearic acid by the usual debromination procedure followed by esterification of the linoleic acid with methanol.

The t,t-10,12,linoleic acid was prepared from dehydrated castor oil fatty acids according to the method of von Mikusch (13). The acid had a melting point of 56-57°C. and was employed for the preparation of the methyl ester.

Blown methyl oleate was obtained by passing air through methyl oleate at a rate of 1 liter per minute at 80-85°C. until the desired peroxide value was obtained.

Methyl 9,10-epoxystearate was prepared from methyl oleate according to the method of Findley *et al.* (5).

Methyl 9,10-dihydroxystearate was obtained by hydrolysis of the epoxy ester.

Dimethyl dilinoleate was furnished by the Northern Regional Research Laboratory through the courtesy of Dr. J. C. Cowan. The ester was prepared from soybean oil and was distilled in a centrifugal molecular still.

Residual methyl dimeric ester was obtained by esterification of commercial "Dimer Acids" manufactured by Emery Industries Inc.

The styrene was a 99% commercial grade. It was distilled prior to use in an all-glass apparatus.

Eastman Kodak White Label benzoyl peroxide was used without purification.

Fischer's C. P. Xylene was shaken with fuming sulfuric acid, washed, dried over anhydrous sodium sulfate, and distilled prior to use.

Polymerization. In a typical experiment 10 g. of styrene and 10 g. of methyl ester were dissolved in 20 g. of xylene. The mixture was refluxed for 16 hours. Generally benzoyl peroxide, in amounts from 1 to 10% by weight based on the styrene, was added as a catalyst.

Separation Procedures. Unreacted styrene and solvent were removed from the reaction mixture in vacuo. The residue was then refluxed for 15 minutes with approximately 10 times its volume of methyl alcohol, and the alcohol was decanted from the insoluble residue. Extraction with methanol was repeated twice. From the combined methyl alcohol extracts a fatty ester fraction I was obtained by evaporation of the alcohol.

The methanol insoluble residue was dissolved in approximately twice its volume of benzene, and the precipitate formed by the addition of about 10 volumes of methanol for one volume of this benzene solution was separated. The benzene methanol solution was evaporated to dryness to recover fraction II. The insoluble residue, dried in vacuum, gave fraction III.

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