A bulk analysis of Osage orange is shown in the following table:

TABLE II

Bulk Analysis of Osage Orange Fruit (Dry Weight)

I. Seed:			40%
A. Hulls		50%	
B. Kernels		50	
1. Oil	42%		
2. Meal	58		
a. Protein	67%		
b. Non-Protein	33		
II. Fruit Bulk			60%

McHargue (12) has reported a rather detailed analysis of the mineral constituents of the Osage orange fruit. He found CaO, 0.16; MgO, 0.20; K_2O, 3.82; Na_2O, 0.13; P_2O_5, 0.67; and total (crude) ash, 6.60% of the dry fruit. The dried fruit contains more nitrogen and more than twice as much potassium as wheat bran (12).

From the standpoint of commercial production the Osage orange holds some remarkable possibilities. The young trees grow rapidly under a great variety of soil and climatic conditions. Actual harvesting measurements have shown that the yield of fruit per tree may vary from nothing to as much as 1,000 pounds or more. The female of the species starts bearing fruit at 4 to 6 years of age. Thereafter the yield increases to a maximum and gradually decreases.

There are no definite indications that any toxic substances are present in dried Osage orange fruit (16, 19). The fresh fruit is said to cause dairy animals to "dry-up," and this is possibly due to the low molecular weight resins present in the latex, causing some inflammatory action in the digestive tract. The fruit has been successfully used as a feed for horses, mules, and steers. It is doubtful if any harmful effects could arise from feeding the dried fruit, for during the drying process the resins become polymerized and quite inert from a physiological standpoint. The boiled latex has been injected subcutaneously into pigeons without any visible effects (8). If the fresh latex is injected in the same manner, the proteolytic enzymes present cause sufficient digestion, *in vivo,* to kill the experimental animal. To our knowledge no cases of poisoning have occurred as the result of feeding Osage orange fruit.

The wide geographical distribution of the Osage orange tree, its adaptability to many types of soils and environmental conditions, its resistance to drought and plant diseases, and the tremendous yields of fruit obtainable makes this tree worthy of consideration as a producer of industrial raw materials, especially in view of the several valuable materials (oil, resins, sugars, pigments, and feed-stuff) occurring in the fruit. In addition, there are several unidentified minor constituents of the fruit which may ultimately enhance its commercial value.

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Component Fatty Acids of Some Cruciferae Oils[1,2,3]

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Brassica seed oils, like rape, mustard, ravison, etc., form a very important group of edible and industrial oils. Considerable investigation on the fatty acid composition of oils of this species has been reported recently by many workers (1, 2, 3). In view of some recent work by Hilditch (1, 2) in which he used low-temperature fractional crystallization methods prior to distillation of the methyl esters, the earlier work of Foreman and Brown (3a) that eicosenoic acid was present in rape seed oil, and the indication by Hopkins (3) that eicosenoic acid was present in the seed fat of a wild species of a cruciferae plant (*Brassica orientalis*), hare's ear mustard, the results of earlier investigations (4, 10) have been considerably revised.

The development of the method for the quantitative determination of highly unsaturated acids by ultraviolet absorption after alkali isomerization (5)

[1] Contribution No. 708 from the Department of Chemistry, University of Pittsburgh.

[2] Presented in part at the Spring meeting of the American Oil Chemists' Society, held in New Orleans, La., May, 1948.

[3] This investigation had been completed prior to the publication of Baliga and Hilditch's paper, "The Component Acids of Rapeseed Oil" (J. Soc. Chem. Ind., *67*, 258-262 (1948).

and the subsequent successful application of this method for the determination of the fatty acid composition of many fats (6, 7) have offered a very simple procedure for fatty acid analyses. This relatively simple method has been applied by Longenecker and Baldwin (6, 7) to the investigation of fats which contained C_{18} acids as the predominant unsaturated acids. Few fats containing higher unsaturated acids have been investigated previously by this method.

The present investigation was undertaken to determine the feasibility of applying the above procedure to the determination of the fatty acid composition of some cruciferae oils, which contain a C_{22} unsaturated acid as their major constituent, and also to investigate the component fatty acids of yellow mustard seed (*Brassica alba*) oil, of Indian origin, the fatty acid composition of which has not been heretofore reported.

Experimental

The yellow mustard (*Brassica alba*) and black mustard (*Brassica nigra*) seeds used in this investigation were supplied through the courtesy of the Government of Punjab, India. The seeds were crushed and extracted with light petroleum ether in a Soxhlet apparatus. The solvent was removed under reduced pressure in an atmosphere of nitrogen and the bright yellow oil obtained in each case was analyzed for various constants. The rapeseed (*Brassica Compestris*) oil, of unknown origin, was purchased from M-S Tunley and Company of New York. The analytical constants of these oils are listed in Table I.

TABLE I

Analytical Constants of Cruciferae Oils

No.	Constants	Yellow Mustard	Black Mustard	Rapeseed
1	Yield of oil in weight of dry seed	102.2	35.6%	.....
2	Iodine value	39.5%	106.2	106.8
3	Saponification equivalent	324.6	323.0	329.6
4	Unsaponifiable	3.8%	3.0%	1.1%
5	Acid value	0.6	0.5	1.2
6	Linoleic acid	14.0%	17.6%	13.9%
7	Linolenic acid	6.9%	6.3%	9.4%

The oils were saponified with alcoholic potassium hydroxide; the soaps hydrolyzed with a mineral acid, and the fatty acids which were liberated were converted into methyl esters by the usual method. These esters were fractionally distilled through an efficient, electrically heated fractionating column, and the various fractions were analyzed for iodine value, saponification equivalent, and the content of octadecadienoic and octadecatrienoic acids. The percentages of diene acid (octadecadienoic) and triene acid (octadecatrienoic) were determined by the method of Mitchell, Kraybill, and Zscheile (5), as modified by the Spectroscopy Committee of the American Oil Chemists' Society. The whole of the unsaponifiable matter was assumed to remain in the residue. This was removed by the usual method, and the various analytical constants of non-saponifiable-free fraction were also determined. From the results of the analyses of each fraction the component fatty acids of the oil were calculated. The tabulated results obtained from the fractionation data for each oil are given in Tables II, III, and IV.

The presence of eicosenoic acid was indicated from the calculated fractionation data of these oils. The methyl ester fractions of rapeseed oil, which from their value of the saponification equivalent indicated the maximum amount of C_{20} acids, were combined together and refractionated to get the maximum concentration of C_{20} acids. The results of this fractionation are shown in Table V.

TABLE II

Fatty Acids of Yellow Mustard Seed Oil as Calculated From the Fractionation Data

Acids	Weight of ester fraction, g.	Weight per cent of esters including unsaponifiable	Weight per cent of acids including unsaponifiable	Weight per cent of acids excluding unsaponifiable
Myristic	0.23	0.4	0.4	0.4
Palmitic	0.79	1.4	1.4	1.5
Stearic	0.23	0.4	0.4	0.4
Arachidic	0.28	0.5	0.5	0.5
Behenic	1.07	1.9	1.9	2.0
Lignoceric	0.56	1.0	1.0	1.0
Oleic	12.00	21.3	21.1	22.0
Linoleic	7.80	13.8	13.7	14.2
Linolenic	3.78	6.7	6.6	6.8
Eicosenoic	3.78	6.7	6.8	7.0
Erucic	23.90	42.4	42.7	44.2
Unsaponifiable	1.95	3.5	3.5	.....
	56.37	100.0	100.0	100.0

Fraction No. 3, which has an iodine number of 79.5 and saponification equivalent of 324.2, seemed to contain a maximum amount of C_{20} acids. The procedure used by Hopkins (3) was followed to confirm the presence of eicosenoic acid in this fraction. This fraction was converted into hydroxy acids by the method of Lapworth and Mottram (8) as follows:

Methyl ester (5 g.) was saponified with concentrated sodium hydroxide solution, and the mixture

TABLE III

Fatty Acids of Black Mustard Seed Oil as Calculated From the Fractionation Data

Acids	Weight of ester fraction, g.	Weight per cent of esters including unsaponifiable	Weight per cent of acids including unsaponifiable	Weight per cent of acids excluding unsaponifiable
Myristic	0.49	0.8	0.8	0.8
Palmitic	0.56	0.7	0.7	0.7
Stearic	.....	.....	.....	.....
Arachidic	0.35	0.5	0.5	0.5
Behenic	1.53	2.2	2.2	2.3
Lignoceric	1.25	1.8	1.8	1.8
Oleic	13.06	20.2	20.1	20.7
Linoleic	12.20	17.6	17.5	18.0
Linolenic	4.38	6.3	6.3	6.5
Eicosenoic	5.42	7.8	7.9	8.1
Erucic	27.30	39.3	39.5	40.6
Unsaponifiable	1.95	2.8	2.8	.....
	69.49	100.0	100.0	100.0

evaporated to a paste. This was dissolved in three liters of distilled water and neutralized with mineral acid. Sodium hydroxide (5 g.) was added and the solution cooled to 15° C. and retained at that temperature during the addition, with stirring, of a 1% potassium permanganate solution (400 ml.). The mixture was stirred for about 15 minutes and then decolorized with sulfur dioxide. It was then acidified with hydrochloric acid (150 ml.). The white, flocculent precipitate of dihydroxy acids was filtered off, drained for a short time, washed with petroleum ether (about 50 ml.) to facilitate drying, then dried in a vacuum desiccator to constant weight. The dried precipitate was extracted in a Soxhlet apparatus with hexane (b.p. 80-90° C.) to remove saturated acids and then with dry ether to remove most of the dihydroxystearic acids. The residue was recrystallized with ethyl acetate, whereupon a product was obtained which melted at 128-129° C. Dihydroxy acids were

TABLE IV

Fatty Acids of Rapeseed Oil as Calculated From the Fractionation Data

Acids	Weight of ester fraction, g.	Weight per cent of esters including unsaponifiable	Weight per cent of acids including unsaponifiable	Weight per cent of acids excluding unsaponifiable
Myristic	1.92	0.6	0.6	0.6
Palmitic	3.20	1.0	1.0	1.0
Stearic	0.65	0.2	0.2	0.2
Arachidic	1.28	0.4	0.4	0.4
Behenic	8.02	2.5	2.5	2.5
Lignoceric	3.84	1.2	1.2	1.2
Oleic	56.00	17.5	17.4	17.6
Linoleic	43.50	13.6	13.5	13.7
Linolenic	28.80	9.0	8.9	9.0
Eicosenoic	10.40	3.0	3.0	3.0
Erucic	158.60	49.9	50.2	50.8
Unsaponifiable	3.52	1.1	1.1	....
	319.71	100.0	100.0	100.0

also prepared by the above procedure from pure specimens of oleic acid (m.p. 13°C.) and erucic acid (m.p. 34.3°C.). These dihydroxy acids melted at 132 and 130°C., respectively.

TABLE V

Refraction Data for Rapeseed Oil Methyl Esters

No.	Fraction weight, g.	Iodine value	Saponification equivalent
1	2.20	145.2	249.0
2	2.18	116.1	302.5
3	5.60	79.5	324.2
4	3.92	71.5	343.6
5	8.10	71.0	347.6

When the unknown substance of m.p. 128-129°C. was mixed with dihydroxystearic acid (m.p. 132°C.), the mixture melted at 123-125°C. When it was mixed with dihydroxybehenic acid (m.p. 130°C.), it melted at 123-125°C. Therefore, it was concluded that this substance was not a dihydroxy acid obtained from oleic or erucic acids. Moreover, it behaved as a single substance as evidenced by the constancy of the melting point after repeated fractional crystallization. The dihydroxy acid obtained from Fraction 3, Table V, when analyzed, gave the following constants: I. V. =0; Sap. Equiv. = 345.0. Calculated for the dihydroxyeicosanoic acid, I. V. = 0; Sap. Equiv. = 347.0.

The dihydroxy acid melting at 128-129°C. was converted into semicarbazones by the method used by Baldwin and Parks (9). The alkali-soluble semicarbazone obtained melted at 155°C. (Hopkins reported 156-157°C.), and when this semicarbazone was mixed with the corresponding semicarbazones from dihydroxystearic and dihydroxybehenic acids, it melted as follows:

With alkali-soluble semicarbazone (m.p. 162°C.) from dihydroxystearic acid, it melted at 142°C.

With alkali-soluble semicarbazone (m.p. 160°C.) from dihydroxybehenic acid, it melted at 144°C.

Analysis of the alkali-soluble semicarbazone of the unknown dihydroxy acid gave the following results:

Calcd. for $C_{12}H_{23}O_3N_3$, N, 16.3%.

Found: N, 15.95%.

It is therefore concluded from the above results that the C_{20} monoethenoid acid present in rapeseed oil is probably 11:12 eicosenoic acid.

The above procedure for confirming the presence of eicosenoic acid was also followed in the case of mustard seed oils, but a sufficient concentration of C_{20} unsaturated acid could not be obtained in one single fraction, and the presence of eicosenoic acid could not be confirmed in these oils.

Discussion

The results of the present investigation along with those determined by other methods for the fatty acid composition of various cruciferae oils are summarized in Tables VI and VII. From inspection of the data in these tables it may be seen that the results obtained by the method used in the present investigation compare favorably with those reported by other methods. However, just as previously reported for rapeseed oil (2), the results for the mustard seed oils differed in two important respects from the previous investigations (4, 10). The amount of linolenic acid found was considerably higher than that previously reported for these oils, and the presence of eicosenoic acid, not heretofore reported, was also indicated. The presence of a two double-bond unsaturated acid which had been reported by Hilditch and co-workers (1, 2) to be present in rapeseed oil could not be established from spectral and other analytical data.

TABLE VI

Comparison of Fatty Acid Composition of Rapeseed Oils

Acids	Indian Hilditch, *et al.* (4)	Indian Hilditch, *et al.* (1)	Indian Hilditch, *et al.* (2)	Unknown Origin This investigation
Myristic	.....	0.2	.....	0.6
Palmitic	2.0	2.2	1.9	1.0
Stearic	.....	0.4	3.5	0.2
Arachidic	.....	0.5	0.7	0.4
Behenic	.....	1.5	0.7	2.6
Lignoceric	1.0	0.8	0.8	1.2
Oleic	17.0	15.2	12.3	17.7
Linoleic	29.0	16.0	15.8	13.8
Linolenic	.....	7.0	8.7	9.1
Eicosenoic	.....	.....	4.8	3.1
Erucic	51.0	53.8	47.8	50.4
Hexadecenoic	.....	0.1	1.5	.....

From the inspection of the data in Table VII it may be seen that the nature of fatty acids in the yellow mustard seed oil of Indian origin does not much differ from those found in the other cruciferae seed oils. This oil like other *Brassica* oils contains C_{18} and C_{22} acids as the major unsaturated acids.

TABLE VII

Comparison of Fatty Acid Composition of Mustard Seed Oil

Acids	Hilditch, *et al.* (4) Black mustard (English)	Sadborough, *et al.* (10) Black mustard (Indian)	This Invest. Black mustard (Indian)	Hilditch, *et al.* (4) White mustard (English)	This Invest. Yellow mustard (Indian)
Myristic	.....	0.5	0.8	.....	0.4
Palmitic	2.0	.....	0.7	2.0	1.5
Stearic	trace	.....	.....	trace	0.4
Arachidic	trace	.....	0.5	1.0	0.5
Behenic	.....	3.8	2.3	.....	2.0
Lignoceric	2.0	1.1	1.8	1.0	1.0
Oleic	24.5	32.3	20.7	28.0	22.0
Linoleic	19.5	18.1	18.0	14.5	14.2
Linolenic	2.0	2.7	6.5	1.0	6.8
Eicosenoic	.....	.....	8.1	.....	7.0
Erucic	50.0	41.5	40.6	52.5	44.2

Summary

The oils from yellow mustard seed (*Brassica alba*), black mustard seed (*Brassica nigra*) of Indian origin, and rapeseed (*Brassica Compestris*) of unknown origin have been analyzed for their fatty acid composition without preliminary resolution of fatty acids by lead-salt-alcohol or fractional crystallization methods. The results compare very favorably with those determined by other recently developed methods. It may be concluded therefore that this method can be favorably employed for the determination of

fatty acid composition of fats containing higher unsaturated acids.

Confirmatory evidence has been obtained for the presence of eicosenoic acid in rapeseed oil.

The nature and amount of fatty acids of yellow mustard seed oil of Indian origin do not differ in any significant manner from those of other cruciferous seed oils.

The present analysis of black mustard seed oil reveals a higher amount of linolenic acid, and the presence of a C_{20} monoethenoid acid, not heretofore reported.

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Stabilization of Vitamin A in Halibut Liver Oil With Nordihydroguaiaretic Acid (NDGA)[1]

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THE importance of adequate vitamin A in the diet is generally recognized. Certain fish liver oils, being especially rich in vitamin A, have been used for many years as a dietary supplement in American households. Halibut liver oil is one of the vitamin A oils in popular use today, but, in common with other fish liver oils, it is a relatively unsaturated oil and is susceptible to rancidification if exposed to air. It has been shown by Hilditch (1) and also by this study that the oxidation of the fresh fish liver oil to a rancid oil is accompanied by destruction of a large share of the vitamin A content. Exposure to light and warm temperatures accelerates the rate at which the freshness of the oil is lost. Most purified fish liver oils or concentrates tend to become rancid if the oil is dispensed from an open container and stored at room temperature.

To ensure the maximum freshness of the oil at the time of purchase, special precautions are taken in the commercial production, purification, and packaging of the vitamin A oil to minimize exposure to oxidizing conditions. Natural antioxidants present in the oil are important in the subsequent preservation of the freshness of the oil during the consumption period. Further stabilization of the oil may be obtained by the addition of more effective antioxidants.

Since purification and concentration of the vitamin A oil by alkali processing tends to remove natural antioxidants, the addition of antioxidant to either the purified oil or to the concentrate is highly desirable if they are to be dispensed from an open container. A good antioxidant for use in food products should be non-toxic, highly effective in low concentrations, moderately priced, and easily soluble in the product. It should produce no adverse change in the color, taste, or odor of the product. These experiments determined the effectiveness of the chemical, nordihydroguaiaretic acid (NDGA), as an antioxidant in halibut liver oil.

NDGA was developed as an antioxidant at the University of Minnesota in 1944 (2). It is produced as a pure crystalline powder from a common desert plant, one of the creosote bushes, which otherwise is quite useless.

NDGA is one of the group of polyphenolic compounds, characterized by either the ortho- or para-oxygen linkage, which are known to possess primary antioxygenic properties. According to Mattill (3), all other substances which inhibit the autoxidation of fats are properly called synergists because they merely reinforce the effect of the primary phenolic antioxidant present.

NDGA has been shown to be non-toxic in low concentrations and is soluble in hot oils at 75-125°C., ethyl alcohol, propylene glycol, and other organic solvents (2, 4). It may be incorporated easily in any fatty product by dissolving the proper amount of antioxidant in a carrier solvent, which is then mixed in with the bulk of the product. Bucher (5) noted that a 0.1-0.2% suspension of NDGA in salmon oil proved effective in retarding the development of rancidity. A report by Silver (6) showed that application of 0.2% NDGA in a coating of vegetable oil would extend the storage life of brined mackerel from 10 days for the control to 4 months for treated fish. Higgins and Black (7) found that high storage temperatures decreased the stabilizing effect of NDGA in lard.

Experimental

Crude halibut liver oil was obtained from a local extraction plant for the stability tests. The samples were blended from several lots at that plant, and the peroxide values and free fatty acid content were uniformly low. The standard Swift stability method as modified by Riemenschneider (8) was used for all tests at 97.7° C. and 80° C. The antioxidant was dissolved in ethyl alcohol, and one milliliter of the solvent containing the proper concentration for 20 grams of oil was added to the oil at the beginning of the test. The solvent evaporated easily at the temperature of the oil and was carried out of the oxidation tube in a few minutes by the current of air.

Approximately 0.25-gram samples of oil were removed at regular intervals from each tube for the determination of peroxide values and spectrophotometric determination of vitamin A. Duplicate tubes

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