The Composition and Properties of Ragweed Seed Oil

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The present need for large quantities of fats and oils in the United States has stimulated interest in the investigation of new sources of these materials. Microchemical tests on the seed of the common ragweed (Ambrosia eliator) by Pammel and Dox (1) showed that these seeds contain large amounts of fat. However, no detailed investigation of this oil has been made in spite of the fact that the ragweed grows very abundantly in the United States. Its seed could become available in large quantities both by direct harvesting of the seed in stubble fields and by separating ragweed seed from some commercial seed during the cleaning process.

In this investigation the oil was extracted from ragweed seed by petroleum ether. The physical and chemical properties were determined and the fatty acid distribution investigated by means of the ester distillation method.

A material resembling a wax which was separated from the oil, was investigated to a limited extent. A study of the sterols present in the oil was also made.

Experimental

The ragweed seed used was harvested from stubble fields with a combine by the Agricultural Engineering Department of Purdue University. A yield of approximately ten bushels per acre was obtained. After recleaning by a commercial firm, these seed were about 95 per cent ragweed and 4 per cent velvet leaf. A proximate analysis gave the results shown in Table I.

TABLE I

Proximate Analysis of Ragweed Seed

	Moisture *	Ash *	Protein *	Crude Fat *	Crude Fiber *
With hulls.....	5.94	3.07	22.62	18.31	36.35
Hulls removed..	5.75	2.74	24.12	19.33	37.60

* Per cent original weight of seeds.

These results show that ragweed seeds have approximately the same oil content as do soybeans; the protein content is considerably lower but the oil meal from ragweed seed would be definitely classed as a protein concentrate.

The oil was obtained by extracting the ground seed with petroleum ether in a laboratory Soxhlet extraction apparatus. The solvent was removed by bubbling nitrogen through the oil under reduced pressure.

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A small amount of oil was also obtained by pressing the seed in a Carver hydraulic press. The chemical and physical properties of both the extracted and expressed oils are shown in Table II.

Both the iodine and thiocyanogen values are higher than those usually found for soybean oil. Therefore, the ragweed seed oil might be expected to have drying properties somewhat superior to those of soybean oil.

Slight differences in the values for the expressed and extracted oils are due to the presence in the extracted oil of rather large amounts of some solid material resembling a wax.

Preliminary experiments on the drying rate of ragweed seed oil indicate that it reaches the tacky state in only about two-thirds the time required by soybean oil, and, like the latter oil, it does not form a hard film under the experimental conditions used. The same relative superiority over soybean oil was exhibited by ragweed seed oil in heat-bodying experiments.

Fatty Acid Composition of Ragweed Seed Oil

For the determination of the individual fatty acids 100 grams of extracted ragweed seed oil were saponified by boiling with alcoholic potassium hydroxide. The unsaponifiable matter was removed from the soaps by repeated extraction with petroleum ether. The soap solution was then evaporated to dryness by bubbling nitrogen through it while it was being heated under reduced pressure. The soaps were dissolved in water and the fatty acids freed with sulfuric acid and extracted with ethyl ether. The mixed fatty acids had the following properties: iodine No. —146.6; thiocyanogen value—85.9; molecular weight —279.7.

In separating the saturated from the unsaturated fatty acids, a comparison was made between the modified Twitchell method and the crystallization method described by Earle and Milner (2). The latter method was found to be superior in both the yield and purity of the saturated acids obtained. Therefore, the crystallization method was used both for the quantitative determination of the saturated acids present and for the separation of larger quantities to be distilled as esters. The mixed fatty acids were found to consist of 10.3 per cent saturated and 89.7 per cent unsaturated acids.

After removal of the unsaturated acids, the material soluble in acetone at —40° was recovered and

TABLE II

Chemical and Physical Properties of Ragweed Oil

	Unsap. Matter (%)	I_2 No.	Sapn. No.	T. V.	Acid No. (% F.F.A.)	Sp. gr. 25°/25	Viscosity (poises)	R. I.
Expressed oil.....	1.22	141.5	191.2	.....	2.98	0.9214	0.52	.....
Extracted oil.....	1.84	140.7	189.2	81.0	3.17	0.9210	.....	1.4686

used as the unsaturated acid fraction. The properties of these two fractions are given in Table III.

TABLE III

Summary of Chemical Properties of Mixed, Unsaturated, and Saturated Fatty Acids

	Mean Mol. Wt.	I_2 No.	T. V.
Mixed F. A.	279.7	146.6	85.9
Unsatd. F. A.	282.5	162.7	95.3
Satd. F. A.	269.4	1.4	.....

The mean molecular weight of the unsaturated acids indicate that they are largely C_{18} acids and the iodine and thiocyanogen values definitely show that these acids contain large amounts of linoleic and very little linolenic acid.

The saturated acid fraction seems to be made up of nearly equal amounts of palmitic and stearic acids.

The saturated and unsaturated acid fractions were esterified separately by boiling for three hours with five times their weight of methanol containing 2 per cent sulfuric acid. The esters were washed in the usual manner.

Distillation was carried out in a fractionating column containing a distilling tube 15 mm. in diameter and 33 inches in length. The column was packed with wire gauze of the type designed by Ewell and Lecky (3). The pressure during fractionation of the esters was approximately 1 mm.

TABLE IV

Fractionation Data for Saturated Fatty Acid Esters
Original charge—31.34 g. saturated esters

Fraction	Wt. Fract. (g.)	Distn. Temp. °C.	Mean Mol. Wt.	Per cent Palmitic Ester	Per cent Stearic Ester
1	4.25	132	268.3	100.0	.....
2	9.21	132	269.2	100.0	.....
3	3.71	133-141	273.0	91.6	8.4
4	5.70	142-145	298.3	.....	100.0
Residue (+holdup)	8.28	.....	317.0	.....	94.2
Totals	31.15			55.0	45.0

Fractions 1 and 2 are assumed to be pure palmitic acid, fraction 3 to be a mixture of palmitic and stearic acids, and fraction 4 to be pure stearic acid. The high molecular weight of the residue was apparently due to the presence of some of the original unsaponifiable matter of the oil rather than to a high molecular weight fatty acid. This assumption was proved to be true by the isolation of unsaponifiable matter from the soaps of the residue. Therefore, this fraction was assumed to consist of stearic acid and inert material and calculations were made on that basis. From these calculations it was found that the saturated fatty acid fraction consists of 54.8 per cent palmitic and 45.2 per cent stearic acid.

The free acids were recovered from fractions 2 and 4 and were recrystallized from acetone. Melting points and mixed melting points, made with known samples of palmitic and stearic acids, showed that fraction 2 was pure palmitic acid and that fraction 4 was pure stearic acid.

The fractionation data for the unsaturated acid esters are shown in Table V.

The mean molecular weights and distillation temperatures of these fractions indicate that only C_{18} acids are present. The slightly higher mean molecular weight of the residue is probably due to a small amount of polymerization. Calculations from the iodine and thiocyanogen values show that linolenic acid is present only in traces if it is present at all. Therefore, the acid distribution was calculated from the iodine numbers since they can be determined somewhat more accurately than the thiocyanogen values.

The fatty acid distribution of the unsaturated fraction was also calculated from the iodine numbers and thiocyanogen values of the mixed fatty acids and the unsaturated fatty acids before esterification. The amount of linoleic and linolenic acids present was determined by the use of spectroscopic method* (4) which makes use of the fact that both of these acids form conjugate systems when treated with alkali.

The results in Table VI show reasonably good agreement between the different methods.

TABLE VI

Comparison of Unsaturated Acid Distribution by Different Methods

Method	Oleic (Wt.%)	Linoleic (Wt.%)	Linolenic (Wt.%)
Ester fractionation	22.2	77.8	....
Calcd. from mixed fatty acids	20.5	78.5	1.0
Calcd. from unsat. fatty acids	20.6	79.0	0.4
Spectroscopic	.....	74.1	1.1

For proof of the presence of the individual unsaturated fatty acids, the remaining esters of fractions 1, 2, and 4 were combined and the free acids were liberated. These acids were then brominated according to the method of Eibner and Muggenthaler (5). No hexabromostearic acid was found but this fact does not exclude the presence of small amounts of linolenic acid. The ethyl ether was removed and the residue crystallized from petroleum ether. A good yield of tetrabromostearic acid (m.p. 115°) was obtained. The filtrate was evaporated to dryness, debrominated, and the free acids recovered. These acids were oxidized according to the combination of methods used by Sullivan and Bailey (6). The hydroxystearic acids formed were filtered and extracted with ethyl ether in a Soxhlet apparatus. The ether soluble portion was recrystallized twice from 95 per cent ethanol. Its melting point of 130° indicated that it was dihydroxystearic acid.

* This determination was made by Dr. F. P. Zscheile of this laboratory.

TABLE V

Fractionation Data for Unsaturated Fatty Acid Esters
Original charge—49.05 g. unsaturated esters

Fraction	Wt. Fract. (g.)	Distn. Temp. °C.	Mean Mol. Wt.	I_2 No.	T. V.	Per cent Oleic Ester	Per cent Linoleic Ester
1	8.60	131-132.5	291.6	160.6	90.4	13.6	87.4
2	9.84	132-133	293.0	162.3	91.6	11.6	89.4
3	15.74	134	290.8	157.8	90.6	16.8	83.2
4	5.59	133	294.3	156.2	90.4	18.7	81.3
Residue (+holdup)	8.92	.....	301.2	125.8	74.8	53.7	46.3
Totals	48.69					22.2	77.8

The similarity in the fatty acid composition of ragweed seed oil and soybean oil is shown in Table VII.

TABLE VII
Fatty Acid Composition of Ragweed Oil and Soybean Oils

Fatty Acid	Ragweed Oil	Soybean Oils
Palmitic	5.5	6.8-14.3
Stearic	4.8	2.4- 5.5
Oleic	19.9	25.9-33.7
Linoleic	69.8	50.7-58.8
Linolenic	(Possible traces)	2.1- 6.5

Wax-Like Constituents of Ragweed Seed Oil

Separation from the oil at room temperature of a white amorphous material gave evidence of an appreciable amount of wax mixture. To remove most of the waxy material 1945 g. of extracted oil were diluted with 2 l. of acetone, and the mixture allowed to stand for three hours at about —10° C. The insoluble portion was removed by suction filtration and twice more stirred with acetone, cooled, and filtered to remove adhering glyceryl esters. A final low temperature crystallization from a carbon tetrachloride-acetone mixture (acetone added to a hot carbon tetrachloride solution of the wax until turbidity occurred) yielded 23 g. of wax, representing an approximate yield of 1.2 per cent of the extracted oil.

Since naturally occurring waxes are in most cases complex mixtures, the ragweed wax was treated accordingly. The mixture was resolved into simpler constituents according to the procedure described by Zweig and Taub (7). The entire wax mixture was saponified and the unsaponifiable fraction removed by extraction with benzene. After liberating the fatty acids from their soaps, they were separated into two fractions according to their solubility in 95 per cent ethanol. When the unsaponifiable matter was treated with a hot amyl alcohol-hydrochloric acid mixture, the hydrocarbons were insoluble. Upon cooling to room temperature the higher alcohols precipitated while the lower alcohols remained in solution.

TABLE VIII
Properties of Fractions of Ragweed Oil Wax

Fraction	Per cent of Entire Wax	M.P. °C.	Ave. Mol. Wt.
Entire wax	....	64-70	.....
Total acids	23	68-73	418
Sol. in cold alc.	4	Completely at 60	331
Insol. in cold alc.	19	72-75	443
Unsaponifiable	77	.....	.....
Hydrocarbons	55	66.3-66.8	419
Alcohols (plus other unsapon. matter)	22	.....	.....
Insol. in cold amyl alc.-HCl	16	75-78	420
Sol. in cold amyl alc.-HCl	6	68-72	.....

The separation of the wax acids on the basis of their solubility in 95 per cent ethanol shows a marked predominance of higher molecular weight acids. With the small alcohol-soluble fraction possessing an average molecular weight near that of a C_{22} acid, the possibility of more than traces of stearic acids and lower homologs is ruled out. The higher melting point and average molecular weight (slightly below that of a C_{20} acid) of the insoluble fraction indicate that most of the wax acids are in the C_{24} to C_{36} acid range.

To secure a pure paraffin fraction for the melting point and molecular weight determinations, the hydrocarbon fraction was heated with concentrated sulfuric acid at 110° C. for two hours with stirring and was then extracted from the diluted acid solution with carbon tetrachloride. This procedure was repeated twice. Crystallization of the paraffin from petroleum ether-acetone (1:1) gave excellent flat lustrous crystals. Considerable work has been done by Chibnall, Piper, *et al.* (8) in determining the constituents of mixtures from their transition and melting points and X-ray crystal spacings. Such treatment was beyond the scope of this work. An approximate molecular weight of 419 was obtained by the Rast method, this weight corresponding with that of C_{30} paraffin. The melting points for mixtures with this average molecular weight agree well with the observed value. Since complex mixtures of paraffins are common in nature, it can only be concluded that the ragweed paraffin is probably a mixture of high molecular weight hydrocarbons.

The wax alcohol fraction insoluble in the cold amyl alcohol-hydrochloric acid mixture was recrystallized twice from acetone and the acetate prepared by refluxing with acetic anhydride. The acetate was crystallized from petroleum ether in the cold and melted from 64-66° C. An approximate molecular weight determination on the insoluble alcohol fraction by the Rast method gave 420, which corresponds to that of a C_{29} alcohol. The almost universal occurrence of complex alcohol mixture in waxes together with the melting point ranges of the insoluble alcohol fraction and its acetate require that this alcohol fraction be considered a mixture of higher alcohols. The soluble alcohol fraction was difficult to purify from a resinous material. By extracting with ethyl ether, an intimate mixture of this fraction and norit, a purified product was obtained. Only the melting point was determined on this fraction, but it did indicate that lower alcohols — those with molecular weights below that of a C_{26} alcohol — could only be present in small amounts. The range of melting again suggests a mixture.

Sterols from Ragweed Seed Oil

Sterol concentrates were obtained in two ways: by the continuous methanol extraction of dewaxed ragweed oil, and from unsaponifiable fraction accumulations. Since methanol also removes considerable fat, the extract obtained by the former method was saponified. By extracting the soap solution with petroleum ether a further concentrate was obtained.

Since the above concentrates are contaminated with any or all of the wax mixture constituents of the oil, an adsorption method of purification was utilized. The dried impure sterol mixture was dissolved in petroleum ether and poured on a packed column of aluminum silicate adsorbent (9). Suction was applied and washing with petroleum ether was continued until no residue remained upon evaporation of a portion of the filtrate. Elution of the sterols was effected with a benzene-petroleum ether solution (1:3) containing 2 per cent of methanol. Washing was continued until all the sterols had been removed from the adsorbent. The solution was evaporated to dryness and the sterols crystallized three times from 95 per cent ethanol. These sterols melted at 134-135° C. and possessed a $[\alpha]_D^{20°}$ of —31° (chloroform, c = 1).

A quantitative yield of sterols from the extracted oil was obtained by extracting four separate 5-g. samples

according to the official method for determining unsaponifiable matter (1). The four extracts were combined to give two equal samples, which were then evaporated to dryness and each dissolved in 50 ml. of 90 per cent ethanol, a sufficient volume to assure solution. A digitonin solution (0.4-g. digitonin in 40 ml. of 90 per cent ethanol) was added to each unsaponifiable fraction and the mixture allowed to stand at room temperature for 24 hours. The crystalline digitonide was filtered on a sintered glass crucible under suction.

TABLE IX
Yield of Ragweed Oil Sterols

Wt. Oil (g.)	Digitonide (g.)	Sterols (g.)	Yield (Per cent by wt. of oil)
10.027	0.1795	0.0460	0.46
10.004	0.1909	0.0489	0.49

The yield obtained corresponds closely to that from soybean oil, suggesting ragweed oil as a potential source of sterols. Since stigmasterol is important in the syntheses of certain sex hormones, the possible presence of this sterol was investigated by brominating (10) the prepared acetates of ragweed sterols. To 0.301 g. of sterol acetate dissolved in 3 ml. of ethyl ether were added 3.8 ml. of a 5 per cent bromine in acetic acid solution. Upon standing for two hours 24 mg. of crystals were filtered. They were recrystallized twice from chloroform-methanol (methanol added to the chloroform solution until turbidity occurred); these plates melted from 190-191.5° C. Further purification was impossible because of a lack of sufficient material. Although stigmasteryl acetate tetrabromide melts slightly above 200° C., the crystals melting from 190-191.5° C. seemed to be impure stigmasteryl acetate tetrabromide or the tetrabromide of a related steryl acetate.

Conclusions

Ragweed seed contains approximately 19 per cent fat and 23 per cent protein. Large quantities of these seed can be readily obtained both from direct harvesting of the ragweed and from the cleaning of some commercial seeds.

The fatty acid distribution in ragweed seed oil is as follows: palmitic acid—5.5 per cent; stearic acid—4.8 per cent; oleic acid—19.9 per cent; linoleic acid—69.8 per cent; linolenic acid—possibly traces. The composition of this oil indicates that it would have slightly better drying properties than soybean oil. The results of preliminary drying and heat-bodying experiments suggest the limited use of ragweed seed oil in paints and varnishes.

No investigation has been made of the edible properties of ragweed seed oil but its relative freedom from linolenic acid indicates its use in the edible field.

Ragweed seed oil contains about 1.2 per cent of a wax mixture which is made up of 55 per cent hydrocarbons, 23 per cent high molecular weight acids, and 22 per cent high molecular weight alcohols. Sterols occur in ragweed seed oil to the extent of 0.48 per cent of the weight of the oil. The unsaponifiable matter also contains high molecular weight hydrocarbons and alcohols. Pure mixed sterols were separated from the accompanying materials by the use of an adsorption process. Bromination of the acetates of the mixed sterols gave evidence for the presence of stigmasterol.

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Determination of Carbon Dioxide in Soap and Soap Products by Loss in Weight

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Introduction

A large number of different forms of apparatus have been suggested for the gravimetric determination of carbon dioxide by loss in weight. The alkalimeter devised by Schrötter * combines simplicity of design with certain advantages that render it particularly suitable for the determination of combined carbon dioxide in soaps. Up to the present time none of the numerous alkalimeters described in the literature have been employed in the analysis of the latter product, their main use being in the analysis of baking powders and limestone. The following paper describes the application of the Schrötter alkalimeter to the analysis of combined carbon dioxide in soaps and soap products. It is quite possible that various of the other types of alkalimeters would prove suitable for use in this determination if it were attempted to employ them.

* Despite a diligent search of the literature the original description of this type of alkalimeter could not be found.

Principle

A weighed amount of the sample is placed in the clean, dry alkalimeter and 5 ml. of 1:2:4 trichlorbenzene added (1) (2). The trichlorbenzene is added to dissolve the fatty acids liberated in the course of the determination thus preventing bumping. Next dilute hydrochloric acid and concentrated sulfuric acid are placed in the appropriate bulbs and the apparatus is weighed. The hydrochloric acid is then allowed to flow down on the sample. The evolved gas passes through the strong sulfuric acid which