enced in checking results by the official method when the samples were washed with equal quantities of alcohol. With the new method check results were readily obtained.

The samples extracted three times in the Soxhlet extractor show approximately the same results as by the official method with a 300 cc. wash. The samples washed six and nine times show still higher results. In practically all samples the solution in the flask becomes cloudy.

From the data obtained there are several sources of possible error in the official method. The results obtained are dependent upon the technique of the analyst; consequently, variable results are obtained by various analysts.

To obtain consistent results, therefore, it is necessary to have a concentration of soap in alcohol of not less than 2.5 per cent. The residue in the filter should not be washed with alcohol, and consequently the free alkali determination should be subordinated from the alcohol insoluble determination. The entire operation should be excluded from CO_2 by some positive prescribed method.

A NON-FATTY OIL FROM JOJOBA SEED*

By R. S. McKINNEY and G. S. JAMIESON
Bureau of Chemistry and Soils, Washington, D. C.

JOJOBA oil is obtained from the seed of Simmondsia californica, Nutt, of the family Buxaceae. The plant is known also as the joboba, while the fruits are known in this country as goat, pig, sheep, or quinine nuts. The shrub is found in Arizona, southern California, and western Mexico, ranging in height from 3 to 15 feet. This thickly branched boxwood-like evergreen shrub is commonly found growing on rocky hillsides. Sheep men in the locality where it grows state "that it is one of the most important of the browsing plants. It is one of the few green things during a dry winter and is eaten freely." It is probably eaten at times also by cattle, pigs and goats. It bears both staminate and pistillate flowers. The fruit is a thin, brown, 3-valved capsule which contains a single seed, having a tough reddish-brown testa. The somewhat egg-shaped seeds average about 0.8 gram in weight but range from .4 to 1.4 grams and are about one-half inch long and three-eighths of an inch in diameter.

For the present investigation a sample of about twenty-five pounds of the seed from the State of Senora, Mexico, was sent to us by L. Kishlar, Manager of Research, Ralston Purina Company, St. Louis, Missouri. These seeds were found to contain 51.2 per cent of oil and 4.7 of moisture. S. Ivanov (Bull. Appl. Botany 32, 129, 1930) reported an oil content of 44.5 per cent for the seed investigated by him.

The seeds were ground in a Hobart mill, and the oil was extracted by cold percolation with petrolic ether. The solvent was removed as far as practicable by distillation at atmospheric pressure. Most of the remaining solvent was removed by heating the oil in an evaporating dish on the steam bath. The last traces were removed at 135° C. under diminished pressure. Over 3 liters of a light yellow limpid oil was obtained.

Table I gives the chemical and physical characteristics of the oil.

TABLE I

Refractive index at 25° C.	1.4648
Specific gravity 25°/25°	0.8642
Iodine number (Hanus)	81.7
Saponification value	92.2
Acid value	0.32
Unsaponifiable matter, per cent	48.3
Iodine number of unsapon. (Rosenmund-Kuhnhenn)	77.2
Acetyl value of unsapon.	171.8
*Saturated acids (Bertram), per cent	1.64
Iodine number (Hanus) — total fatty acids	76.1
Neutralization value of total fatty acids	172.0
Glycerine	0.0

*It should be observed that no separation of the saturated acids could be effected by the lead-salt ether method.

The acetyl value and iodine number of the unsaponifiable matter indicated that it consisted very largely of unsaturated alcohols. A portion of the unsaponifiable matter was brominated at 5° C. and subjected to frictional distillation under diminished pressure, but no saturated alcohols or hydrocarbons were detected. Another portion of about two grams was treated with cold concentrated sulphuric acid. No observable quantity of saturated hydrocarbon separated from this solution upon standing.

About two grams of the unsaponifiable matter was completely hydrogenated using the Adams platinum catalyst, and a 0.90-gram portion of the resulting product was subjected to oxidation with chromium trioxide in a glacial acetic acid solution according to the method of Chibnall and Piper (Biochem. J. 25, 215, 1931). The acids (0.45 grams) were converted into their calcium salts, which were extracted with ether in order to remove any non-acid constituents. The calcium salts were decomposed with hydrochloric acid in the usual manner. The recovered fatty acids after repeated crystallization from alcohol melted at 68.4-69.0°. They gave a neutralization value of 167.5, indicating a mean molecular weight of 335, which is approximately that of behenic acid, but the low melting point indicates that the product is a mixture of acids.

A 100-gram portion of the original oil was saponified by treatment with a solution of sodium ethylate in absolute alcohol. The acids were converted into their calcium salts by treatment with a solution of calcium chloride. The major portion of the unsaponifiable matter separated as an upper liquid phase. This was removed as completely as possible by centrifuging, and further portions were separated by repeated extraction of the remaining calcium salts with ether. The extracted calcium salts were decomposed by treatment with hydrochloric acid, the liberated acids were taken up in ether and the ethereal solution washed with water until free from mineral acid. On removal of the ether 43.2 grams of acids were obtained. This low yield indicated either an imperfect saponification or an incomplete separation of the saponification products. Therefore the unsaponifiable fraction, which had been separated from the calcium

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salts of the acids, was again subjected to saponification for several hours with a strong alcoholic potash solution. The alkaline solution was largely diluted with water and extracted repeatedly with ether. The remaining alkaline solution was acidified with hydrochloric acid, the liberated acids were taken up in ether and the ethereal solution was washed free of mineral acid with water. On removal of the ether 10.3 grams of acids were obtained. The neutralization value, 158.5, of these acids indicated that their mean molecular weight was considerably higher than that of the first portion of acids separated. Consequently it appeared advisable to make a separate study of the two acid fractions.

The main portion of acids (43.2 g.) was further purified from non-acidic substances by converting them into their potassium salts and extracting the dilute alkaline solution with the ether. About one-half gram of a fragrant volatile oil was separated by this treatment but was not investigated. The acids recovered from their salts were converted into their ethyl esters, which were fractionally distilled under reduced pressure from a Ladenburg flask. The data for the distillation and results of the analysis of the ester fractions are given in Table II.

TABLE II
Fractional Distillation of Ethyl Esters.
39.1 g. of fatty acids yielded 41.9 g. of esters.

Fraction	Pressure mm.	Temperature ° C.	Weight grams	Sapon. value	Neutralization Equivalent of acids
I	3	109, 205-9	7.3	169.0	304.0
II	3	208-9	21.0	163.8	314.7
III	2½	210½-12	9.5	161.3	320.0
IV	2½	216-20	3.2	157.7	327.8
Residue	...	......	1.0	138.9	376.0

The analysis of the first fraction shows that the acids present have a mean molecular weight slightly below that of eicosanoic acid. On account of the wide boiling range of this fraction it was redistilled from a micro-Claisen fractionation flask under reduced pressure. The distillation data of these subfractions and their analyses are given in Table III.

TABLE III
Refractionation of Ethyl Esters in Fraction I
4.7 g. of ethyl esters.

Fraction	Pressure mm.	Temperature ° C.	Weight grams	Sapon. value	Neutralization Equivalent of acids
Ia	4	164-201	1.3	*173.5	*295.3
Ib	4	201-8	2.2	167.9	306.0
Ic	4	208-9	0.95	164.0	314.0
Residue	..	.....	0.20	.....	.....

*Hydrogenated.

Fraction Ia, consisting of 1.3 grams, was hydrogenated using the platinum catalyst. The acids were recovered and fractionally crystallized from 95 and 80 per cent alcohol. The first crop of crystals separated from 95 per cent alcohol melted at 72.8° and had a neutralization equivalent of 303. Several recrystallizations raised the melting point to 74.9,° from which point it could not be raised further. This acid and its ethyl ester (m.p. 42.2°) were examined by Dr. Hendricks of this Bureau with the following results:

"Long spacing of
acid ....44.25±.20 A°
"Long spacing of
ester ....27.30±.10 A°

"These values correspond to quite pure eicosanoic acid, for which Francis, Piper and Malkin (Proc. Roy. Soc. London A 128, 214 (1930) found 44.2 for the acid, and 27.6 for the ester by interpolation."

They found that pure eicosanoic acid melted at 75.2°. A small second crop of acids from 95 per cent alcohol, having a neutralization equivalent of 288, consisted of a mixture of stearic and eicosanoic acid. The first crop of acid crystals from 80 per cent alcohol melted at 58.0° and had a neutralization equivalent of 273, corresponding to the 50-50 molar eutectic mixture of stearic and palmitic acid found by Shriner, Fulton and Burk (J.A.C.S., 55, 1494, 1933) to melt at 57.5° and to have a neutralization equivalent of 270.3. The second crop of acid crystals from 80 per cent alcohol melted at 53.8° and had a neutralization equivalent of 262, corresponding to the 70-30 molar eutectic mixture of palmitic and stearic acid which was shown by these investigators to melt at 53.6° and to have a neutralization equivalent of 264. It was concluded that fraction Ia consisted of esters of palmitoleic, oleic and eicosanoic acids.

Ester fraction Ib was saponified, and the resulting fatty acids were recovered in the usual manner. About two grams of these acids were hydrogenated using the Adams platinum catalyst, and the resultant saturated acids were fractionally crystallized from 95 and 80 per cent alcohol. The main crop of acid crystals from 95 per cent alcohol melted at 72.4 and had a neutralization equivalent of 305. After repeated crystallization from alcohol these acids melted at 75.4°, and the melting point was not lowered when the crystals were admixed with an equal quantity of pure eicosanoic acid. The other crops of acid crystals separated from the fraction had lower melting points than the main crop, but their neutralization equivalents were all found to be intermediate between those calculated for stearic and eicosanoic acid. It was therefore concluded that fraction Ib consisted of the esters of oleic and eicosanoic acids. The saponification equivalent of fraction Ic was found to be practically identical with that of fraction II, rendering unnecesary a separate study of this fraction.

About two grams of the esters of fraction II were hydrogenated using the Adams platinum catalyst, and the saturated products were saponified. The alkaline solution was acidified with hydrochloric acid, and the liberated fatty acids were recovered in the usual manner and fractionally crystallized from alcohol. The first crop of crystals, which constituted the main portion of the sample, melted at 72.0° and had a neutralization equivalent of 312. By repeated crystallizations from alcohol the melting point of the acids was finally raised to 74.0°. For comparison, pure stearic and behenic acids were prepared. The stearic acid from the Eastman Kodak Company after being recrystallized several times from alcohol melted at 69.8° C. The behenic acid was prepared from erucic acid by hydrogenation using the Adams catalyst. The resulting behenic acid after repeated recrystallization from ethyl acetate melted at 79.8° C. Francis, Piper and Malkin (loc. cit.) found "that pure stearic acid melts at 70.1°, while pure behenic acid melts at 80.0°. An equal molar mixture of these two acids melted at 73.4° while an equamolar mixture of C_{18}, 20 and 22 acids melted at 66.0°." When the acids separated from fraction II were mixed with pure eicosanoic acid in the proportion of 2 to 1, the resultant mixture melted at 74.4°. It was concluded that the acids separated from fraction II after hyrogenation consisted of eicosanoic acid contaminated with a small amount of another substance shown later to be behenic acid.

1.5 grams of the esters of fraction IV were hydrogenated, the saturated esters saponified and the fatty acids recovered in the usual manner. The fatty acids were taken up in alcohol, decolorized by digestion with Norit and fractionally crystallized from alcohol. The main crop of crystals melted at 73.2° and this melting point could not be raised by recrystallization. These acids, with a neutralization equivalent of 328, were considered to be a mixture of eicosanoic and behenic acids. The melting point of a mixture of equal quantities of these crystals and pure behenic acid was 77.2°, indicating that behenic acid is the principal constituent.

The saponification value of the undistilled residual ethyl esters by a semi-micro-method was 139. The remaining portion of the residual esters, weighing 0.9 gram, was hydrogenated. About 0.4 gram of the hydrogenated product was difficultly soluble in ethyl acetate. An examination of this material revealed that it was composed largely of unsaponifiable matter (alcohols). The presence of this material in the residual esters accounts for their low saponification value. The portion soluble in ethyl acetate was saponified, and the fatty acids were purified by the exhaustive extraction of their potassium salts with ether to remove any traces of alcohols present. The fatty acids were recovered in the usual manner and fractionally crystallized from alcohol. The main crop of crystals melted at 71.0°, and this melting point could not be raised by repeated crystallization from alcohol. The neutralization equivalent of 325 corresponds approximately to an equimolar mixture of eicosanoic and behenic acids. Francis, Piper and Malkin (loc. cit.) have shown that such a mixture melts at 70.8°. When a portion of these crystals (m.p. 71.0°) was admixed with an equal quantity of pure behenic acid the melting point was raised to 76.4°, and when a portion of the crystals was mixed with half as much pure eicosanoic acid the melting point was lowered to 68.8°. Piper, Chibnall and Williams (Biochem. J., 28, No. 6, p. 2181, 1934) have indicated that the molar-melting point curves for binary mixtures of the higher fatty acids are similar. On such an assumption, the raising and lowering of the melting points of these acids are such as would be expected for a 50-50 molar mixture of eicosanoic and behenic acids. It was concluded that fractions II, III and IV, as well as Ic and the undistilled residue, consisted of mixtures of esters of eicosanoic and dicosanoic acids.

The second portion of fatty acids (10.3 g.) separated from the unsaponifiable matter of the oil was converted into their ethyl esters in the usual manner, and the resulting esters were distilled under diminished pressure from a small Claisen flask equipped with a fractionating column. The data for distillation and analysis of the ester fractions are given in Table IV.

TABLE IV

Ethyl Esters of Second Portion of Fatty Acids.

Fraction	Pressure mm.	Temperature ° C.	Weight grams	Sapon. value
I	1½	155-181	2.9	165.5
II	1½	181-6	4.4	151.3
Residue	...	.....	1.9	113.0

An examination of the saponified products of these ester fractions revealed the presence of appreciable quantities of unsaponifiable matter, which in the residue amounted to approximately a third of its weight. The presence of this material accounted for the low saponification values of these esters. After removal of this unsaponifiable matter a portion of fractions I and II were hydrogenated using the Adams platinum catalyst. The hydrogenated acids in each case were crystallized from ethyl alcohol. The main crop of crystals from fraction I melted at 71.0° and had a neutralization equivalent of 326. The major crop of crystals from fraction II melted at 70.8° and had a neutralization equivalent of 324. These values agree closely with those of a 50-50 molar mixture of eicosanoic and behenic acids (mean mol. wt. 326. M.P. 70.8°).

The unsaponifiable fraction (49.6 g.) separated from the 100-gram portion of jojoba oil was refluxed for several hours with a large excess of acetic anhydride. The unreacted acetic anhydride was decomposed with water, and the separated alcoholic acetates were washed until free from acetic acid. The dried acetates (51.9 g.) were fractionally distilled under diminished pressure, with the assistance of R. W. Riemenschneider, Bureau of Animal Industry, United States Department of Agriculture, using his specially constructed electrically heated column (J. Biol. Chem. 113, No. 1, p. 222, 1936). The data for the distillation and the analysis of the acetate fraction are given in Table V.

TABLE V

Fraction	Pressure mm.	Temperature ° C.	Weight grams	Sapon. Value	Refract. Index 25° C.	Iodine Number (Hanus)	Mean Mol. Wt. of Alcohols
I	1	120-54	0.75	263	1.4450	10.5	171
II	1	155-65	3.44	174.0	1.4514	65.8	280.5
III	1	166.8	10.28	163.5	1.4520	72.5	301.0
IV	⅔	168-70	7.96	160.0	1.4521	73.2	308.8
V	⅔	170-5	2.51	157.8	1.4526	68.6	313.7
VI	⅔	175-7	6.89	154.8	1.4532	68.3	320.5
VII	⅔	177	3.40	153.5	1.4533	67.3	323.8
VIII	⅔	177-8	5.17	153.2	1.4534	65.5	324.3
IX	⅔	188	5.07	161.5	1.4570	57.7	305.4
Residue	..	...	4.00	.....	1.4715	60.7	.....

From the very low iodine number of fraction I, it is evident that it contains a considerable amount of saturated constituents. It was not possible, however, to separate any crystalline saturated alcohol from this small fraction. An attempt to prepare the 3.5-dinitrobenzoyl derivatives of the alcohols present was unsuccessful, owing to the products being liquids. As no constituents could be identified this small fraction was not taken into account in the calculations of the proportions of the alcohols present in the oil.

The analysis of fraction III corresponds closely to that calculated for eicosenyl acetate, $C_{20}H_{39}O.C_2H_3O$ (mol. wt. of alcohol 296; iodine number 75.1), while that of fractions VII, VIII and IX correspond to that calculated for dicosenyl acetate, $C_{22}H_{43}O.C_2H_3O$ (mol. wt. of alcohol 324; iodine number 69.4). The unsaturated alcohols recovered from fractions III and IX had refractive indexes of 1.4587 and 1.4623 at 25°. Attempts were made to prepare the alpha napththyl-urethan and the 3,5-dinitrobenzoyl ester of the unsaturated alcohols separated from fraction III and VIII, but in each case the derivatives formed were liquid at room temperature. Portions weighing about 2 grams of fractions II, III and VII of the acetates were hydrogenated. The saturated alcohols were recovered in the usual manner and were fractionally crystallized from alcohol. Two main crops of alcohol crystals were obtained from fraction II, which melted at 67.0° and 66.0°, respectively. Levene and Taylor (J. Biol. Chem. 59, 905-921, 1924) reported that pure synthetic eicosanol melts at 65.5 to 66.5°. Attempts to separate an alcohol of lower molecular weight from this fraction were unsuccessful as were attempts to prepare an alpha-naphthyl-urethan from the alcohol remaining in solution after the separation of the eicosanol described above. The alcohols fractionally crystallized from fraction III melted rather indefinitely at 68.4° and probably consisted chiefly of eicosanol along with some dicosanol. The main crops of crystals separated from fraction VII melted at 72.2°. Levene and Taylor (loc. cit.) reported that pure synthetic dicosanol melts at 70.5-71.5°. The alcohol recovered from fraction X after saponification was found to be solid at room temperature. It was fractionally crystallized from ethyl alcohol, giving crystals which melted sharply at 42.0°. The acetyl value of this alcohol was 118.2, and its iodine number (Rosemund-Kuhnhenn) was 68. The calculated acetyl value for hexacosenol is 117.3 and the iodine number 66.8.

From the results of this investigation of the alcoholic and acidic fractions, the quantities of the constituents identified were calculated. The results are given in Table VI.

TABLE VI

	Per cent
Saturated Acids	1.64
Palmitoleic Acid	0.24
Oleic Acid	0.66
Eicosanoic Acid	30.30
Dicosanoic Acid	14.20
Eicosanol	14.60
Dicosanol	33.70

It has been shown that jojoba oil, from the seed of Simmondsia californica, is not a glyceride fat but a liquid wax, composed almost entirely of esters of high molecular weight, mono-ethylenic acids and alcohols.

The unsaturated acids consist of a mixture of eicosanoic and dicosanoic acids, along with small quantities of palmitoleic and oleic acids. The unsaturated alcohols are a mixture of eicosanol and dicosanol, along with a little hexacosanol and a small quantity of alcohols of lower molecular weight. Its composition indicates that it is somewhat similar to sperm oil.

This oil, on account of its unique composition and properties, could probably be used for several different purposes. When heated to about 300° C. for a short time it becomes colorless. For some months two chemists have used it in place of sulphuric acid in their melting point apparatus.

It would appear useful as an ingredient in prepared waxes, as a lubricant and for the treatment of leather. The sulphonated product might also have some industrial use.

ABSTRACTS

Oils and Fats

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W. F. BOLLENS and M. M. PISKUR

Fat-extraction Apparatus for Feeds. C. E. Weakley, Jr. *Ind. & Eng. Chem.* **28**, 388-389. A description.

Oil Extraction by the Diffusion Process. A. Pfänder. *Fette und Seifen* **43**, 137-9 (1936).—Steam, power and solvent economy are discussed.

Reducing the Oil Content in Cottonseed Press Cake. A. Goldovskiĭ. *Masloboĭno Zhirovoe Delo* **12**, 227-32 (1936).—The successive steps in the extn. of cottonseed oil, such as rolling, roasting and pressing, are discussed. The efficiency of oil extn. depends primarily on the degree of rupture of cell walls. Contrary to American investigators (Thornton, cf. *C. A.* **25**, 1402, 1403, and Woolrich and Carpenter, *C. A.* **28**, 225^{1}, 3258^{8}) the breaking down of cells is chiefly effected in rolling of seeds and not during roasting. In the roasting the rupture of cell walls is completed. (*Chem. Abs.*)

Antioxidants and the Autoxidation of Fats. VI. Inhibitols. H. S. Olcott and H. A. Mattill. *J. Amer. Chem. Soc.* **58**, 1627-1630 (1936).—The unsaponifiable lipid fractions of many vegetables and vegetable oils contain compounds which are active antioxidants to lard and which are here named inhibitols. The inhibitols from wheat germ and cottonseed oils may be concentrated by processes of crystallization and distillation similar to those used for obtaining vitamin E concentrates from which the inhibitols have not been separated. The preparation of inhibitol concentrates from palm oil is aided by the destruction by hydrogenation of the accompanying pro-oxygenic carotenoids. Inhibitol concentrates are transparent oils which have resisted crystallization. Some chemical and physical properties are outlined. The inhibitols are destroyed by reagents which attack a hydroxyl group or saturate a double bond. Inactive esters may be hydrolyzed to regenerate the activity. They are resistant to hydrogenation. Chlorine or bromine addition products can be reactivated with zinc and hydrochloric acid. The concentrates have a strong absorption band at 2940 A. roughly proportional to their activity. The inhibitol concentrates have been assayed by an oxygen absorption method. They are shown to be much more effective antioxidants in lard than any edible compounds which have been suggested for use as commercial antioxidants. The inhibitols protect purified fatty acids and esters but do not protect the vegetable oils from which they are obtained.

Action of Microorganisms on Fat. I. Hydrolysis of Beef Fat by Some Bacteria and Yeasts Tolerating Low Temperatures. J. R. Vickery. *J. Council Sci. Ind. Research* **9**, 107-12 (1936).—The lipolytic activity of several strains of Achromobacter and Pseudomonas, and also of asporagenous yeasts, all of which were capable of comparatively vigorous growth on beef fatty tissue store at —1°, was tested on a synthetic medium