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ALFALFA SEED OIL. ALFALFA INVESTIGATION. VI.

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In the course of the investigations carried on in this laboratory on alfalfa, the subject of the composition of the oil in the seeds arose, and that led to the following work:

The alfalfa seeds were procured in the local market and were of a good quality. They were ground in a small hand mill so that all were crushed or cracked open, after which the oil contained in them was extracted. The ground seeds previous to extraction yielded the following analysis:

Ash	3.43%
Ether extract	11.39%
Crude fiber	10.52%
Moisture	6.35%
Carbohydrates, etc	32.43%
Crude protein	35.88%
	100.00%

According to the above analysis about forty pounds of the seeds would be required to yield a sufficient quantity of the oil for the following investigation, and therefore we determined upon a less expensive solvent than ether for the extraction. Gasoline, boiling between 65° and 95°, at 645 mm., pressure was chosen for this purpose. This solvent was found nearly as efficient as ether for the extraction. More precisely, 1.92% less of the oil was extracted by the gasoline than by the ether.

The extraction was carried out in the following manner: The ground seeds were well stirred up with the solvent so that all the particles were wet, and this mash put into long glass tubes. The solvent was then added at the top and allowed to percolate through. In this way the extraction proceeded easily and quickly, the extraction being considered finished when the solvent came through colorless. The gasoline was then distilled off, when the oil passed from the golden yellow color to a chestnut brown. The oil thus obtained was used for the investigation.

The ether extract was yellowish green in color and it is the authors' opinion that the gasoline extract was a purer oil than the ether extract, which must have contained the coloring matter in the seeds as well as the oil.

Properties of the Oil.—The refractive index of alfalfa seed oil is given in the following table:

Temperature.	n.	Temperature.	n.
17°	1.4783	39.9°	1.4695
20°	1.4770	48.9°	1.4664
25.3°	1.4750	59.2°	1.4622
30°	1.4735	69°	1.4587

The oil is a drying oil whose specific gravity was found to be: 0.9117^{24}_{4} and 0.9149^{24}_{24}. Its saponification value, 172.3; iodine value, 154.2; acid value, 2.85; acetyl value, 19.8; Reichert-Meissl value, 0.40; unsaponifiable matter, 4.40%; glycerol (by acetin), 1.97%; saponification value of the acetylated oil, 192.2.

Volatile Acids.—To determine whether or not there were any volatile acids in the oil, about 3 g. of the oil were saponified with alcoholic potash, the alcohol evaporated off, an excess of sulfuric acid added and the mixture distilled until the distillate reacted neutral to litmus. The distillate was filtered, thus separating the soluble volatile from the insoluble volatile acids. The two portions were then titrated with decinormal sodium hydroxide solution, with the following results:

G. oil.	Sol. volatile acids, cc. *N*/10 NaOH.	Insol. volatile acids, cc. *N*/10 NaOH.
2.944	1.42	0.95
2.910	1.22	1.56

From these results it is concluded that there are practically no volatile fatty acids present in the oil and that there are no lactones present in the free acids.

Insoluble Fatty Acids.—The insoluble fatty acids were prepared by saponifying with alkali, liberating the fatty acids and treating with hot water in the usual manner. The result showed that 92.5% of the oil was in the form of insoluble fatty acids, that is from lauric acid upwards. The acids thus obtained had the following characteristics: neutralization value, 191.5; mean molecular weight, 293; saponification value, 189.9; iodine value, 169.5. The bromide test of Hehner and Mitchell[1] yielded 17% of solid bromides.

Unsaturated Fatty Acids.—The free fatty acids were then separated into the liquid and solid acids by the method of Gusserow-Varrentrapp,[2] that is by the lead-salt method. The quantitative experiments carried out on this separation yielded 90.4% of liquid acids and 9.6% of solid acids in the mixture of acids from the oil. The unsaturated acids, thus obtained, had an iodine value of 175.7, while the saturated acids had an iodine value of 8.4.

The unsaturated acids at hand were insufficient for an extended investigation, so a larger quantity was prepared by the method of Tortelli and Ruggeri (L'Orsi 1900, April) which is claimed to yield unsaturated acids of a greater purity than that of the Gusserow-Varrentrapp method. The acids thus prepared gave an iodine value of 187.7, neutralization value 194.4 and a mean molecular weight of 288.6.

To determine the constituents of the unsaturated acids, the method of the formation of the bromides appears to be the simplest, since

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[1] *Analyst*, **1898**, p. 313.

[2] *Ann.*, **27**, 153 (1828); *Ibid.*, **35**, 197 (1840).

Lewkowitsch in both the fourth and fifth editions of his work on Oils, Fats and Waxes, suggests that this may be done. The bromination was carried out in the following manner: About 3 g. of the unsaturated acids were dissolved in 40 cc. ether, to which a little acetic acid had been added, cooled to 5° and then bromine added, drop by drop, until the solution remained a reddish brown. The mixture was then allowed to stand overnight and filtered the next morning, washed with chilled ether, dried in a water oven and weighed. Lewkowitsch[1] recommends to cool to —10°, and this the authors did, but contrary to his statement, a whiter precipitate was obtained by the former method but of a slightly lower melting point (177.2–178.0°). The filtrate was evaporated to free it from ether and digested with low boiling petrolic ether (35–65° at 645 mm.), upon which the residue passed completely into solution but refused to crystallize out, even on standing overnight at approximately zero degrees. The solvent was then evaporated off, the residue dried in the water oven and weighed, yielding the following results:

	G. unsaturated acids.	G. insol. Br derivatives.	% insol. Br derivatives.	Melting point of Br deriv.
Lot 1......	2.186	0.628	28.7	181.5°
Lot 2......	2.111	0.608	28.8	180.8–181.6°

	G. unsaturated acids.	G. sol. Br derivatives.	% sol. Br deriv.
Lot 1......	2.186	2.746	130.1
Lot 2......	2.111	2.980	136.3

Melting point of linolenic hexabromide.............. 180–181

The mean percentage of the insoluble bromine derivatives (28.75) calculated to linolenic acid gives 10.55%, whereas the ether soluble percentage (133.2), calculated as oleic acid, gives 84.85%. Upon the basis that linolenic acid has an iodine value of 274.10, oleic acid 90.07 and linolic acid 181.42, the iodine value of the mixed unsaturated acids should be 105.34, whereas that found was 187.7. Evidently from this, and especially since the same results were obtained in four different experiments and two different samples of unsaturated acids, the bromine derivatives are only good to estimate the linolenic acid, the remaining unsaturated acids being unadapted to the method.

To determine the other acid or acids, recourse was had to the alkaline permanganate oxidation. This was carried out according to Hazura.[2] The weight of the water-soluble hydroxy acid was 2.9 on a 30 g. sample; the water-insoluble, 21 g. The water-soluble was crystallized from alcohol and water, yielding a pure white product which melted at 173.8°, and had a neutralization value of 147.7. This corresponds to isolinusic acid which melts at 173–175° (Hazura), and has a neutralization value of

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[1] 5th Ed., Vol. I, p. 573.

[2] *Monatsh.*, **1887**, 260, *et seq.*

147.6. A previous oxidation of the unsaturated acids was carried out by passing sulfur dioxide gas into the solution, in place of adding sulfurous acid, yielding an acid melting at 196–198° which signifies the normal linusic acid. Thus both the linusic and isolinusic acid can be obtained from the same unsaturated acid, since in the latter case, no linusic was formed, but in the former, linusic acid was the chief product.

The water-insoluble hydroxy acids were extracted with ether, thus giving the ether soluble and ether insoluble acids. 5.3 g. or about 18% of the oil was unoxidized and remained in the ether solution after the hydroxy acids crystallized out upon the evaporation of the solvent. These latter hydroxy acids (0.41 g.), after being crystallized from alcohol melted at 131.2–131.4° and had a neutralization value of 177.7. This corresponds to dihydroxy-stearic acid, whose melting point and neutralization value LeSueur[1] finds to be 131.5–132.0° and 177.6, respectively.

The ether insoluble portion (9.04 g.) was first fractionated by exhausting with 30% alcohol as recommended by Meyer and Eckert[2] with the following results:

Fraction.	Melting point.
1	156–7°
2	167.6–168.0°
3	173.6–173.8°

Fraction 3, indicates sativic acid since Hazura gives 173° and Hehner and Mitchell 174° as its melting point. Nevertheless, Fraction 3 was again exhausted with water, yielding four fractions with the following melting points: Ist, 173.4–174.2°; 2nd, 177.2°; 3rd, 177.8°; and 4th, 177.6°. The third and fourth fractions were combined and yielded a neutralization value of 161.0 (theoretical for sativic acid 161.2). This is the highest melting point yet recorded for sativic acid, although Hartley[3] obtained 175° and Thoms[4] 177° as the melting point of sativic acid obtained from telfairic acid. This increase in the melting point raised the suspicion that the other fractions (*i. e.*, 1 and 2) must contain another acid, and so they were exhausted with boiling water, with the following results: Fraction 2 yielded five subfractions of different melting points, namely: Ist, 160.6° and neutralization value 159.6; 2nd, 166.4°; 3rd, 171.4°; 4th, 174.6°; 5th, 176.0°. As can be seen, the neutralization value speaks of no second acid present, so the acid must be isomeric with that found in Fraction 1. This is shown to be true by exhausting Fraction 1 with boiling water, yielding the following results:

[1] *J. Soc. Chem. Ind.*, **1900**, 845.

[2] *Monatsh.*, **31**, 1243 (1910).

[3] *J. Physiol.*, **38**, 367 (1909).

[4] *Z. angew. Chem.*, **1904**, 1483.

Fraction	1	2	3	4	5	6
M. p.	158.2	158.0	158.3	158.3	159.2	159.4
Fraction		7	8	9	10	11
M. p.		158.6	158.4	159.2	158.7	159.1

Fractions 1, 2, 3 and 4 were combined and gave a neutralization value of 159.3, Fractions 5 and 6 combined gave 160.7 and Fractions 7, 8, 9, 10 and 11 combined gave a neutralization value of 159.3. In addition, a determination of carbon and hydrogen on the first four fractions mixed, gave 62.08% C and 10.06% H. Theoretical for tetrahydroxystearic acid: 62.02% C and 10.42% H.

Thus, in the first fraction from the oxidation products of alfalfa seed oil, there seem to be two, and possibly three different tetrahydroxy acids present with almost the same melting points (158.2°, 159.4° and 158.8°), but with considerably different solubilities in water, the first fractions yielding granular and amorphous powders while the last are silky crystals.

The oxidation products have therefore revealed the presence, in alfalfa seed oil, of oleic, linoleic and linolenic acids in the proportion of 3.3, 73.2 and 23.5%, respectively, but calculating from the bromine derivatives and the iodine value, they are present to the extent of 3.94, 85.51 and 10.55%, respectively. The former values are considered the more trustworthy.

Saturated Fatty Acids.—The solid saturated fatty acids were obtained by the lead salt separation of the mixed fatty acids, and were purified to a perfectly white mixture by crystallization of the acids from alcohol and the judicious addition of water. After trying several proposed methods for the separation of the saturated acids, the fractional crystallization from alcohol was the method adopted as being the most accurate and expeditious.

The saturated acids gave a neutralization value of 207.7 and a mean molecular weight of 270.2. The melting point was 51.2° and solidifying point 48.8°. About 9.6 g. of the saturated acids were taken and crystallized from 100 cc. absolute alcohol. The first fraction of the crystallized acids had a melting point of 65.8–66.2°, and a neutralization value of 151.0. This fraction was recrystallized three times, the last two subfractions melting at 81.2–81.5° and 83.8°, respectively. The acids from Fraction 1 were then liberated from their potassium soaps by hydrochloric acid, washed and crystallized from 90% alcohol. The first subfraction of this crystallization melted at 74.2° and the second subfraction at 73.8–74.0°.

Judging from the previously obtained neutralization value, either carnaubic or lignoceric acids were present. The melting point of carnaubic acid is 74° according to Meyer and Eckert,[1] but 72.5° according

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[1] *Monatsh.*, 31, 1233 (1910).

to Stürcke.[1] Dunham and Jacobson[2] found carnaubic acid to melt at 72.0–72.5°. Since lignoceric acid melts at 80.5°, carnaubic acid ($C_{24}H_{48}O_2$) must be the one present in alfalfa seed oil.

From the two sets of melting points of carnaubic acid, obtained by different investigators, it would appear that this acid exists in two metameric forms. The high melting points obtained upon Subfraction 1 and 2, from this fraction, would indicate the presence of behenic acid ($C_{22}H_{44}O_2$), but not enough material was at hand to establish this point.

The crystallization was carried further by cooling the solution and the final fractions obtained by the addition of small quantities of water until ten fractions in all were separated. Fractions 9 and 10 melted at 59.6° and 59.2°, respectively. Fraction 9 had a neutralization value of 207.5 The last five fractions were combined and again fractionally crystallized from 70% alcohol, yielding four subfractions melting, in order, at 56.6°, 58.0°, 59.6° and 56.6°, the last being obtained by the addition of a large quantity of water. The first fraction melting at 56.5° was again fractionally crystallized, yielding three subfractions melting from 57.2° to 59.8°. The fraction melting at 57.2 was again recrystallized from alcohol by adding water without change in melting point. It had a neutralization value of 207.5.

From this it is concluded that an acid having a melting point of 59.6–59.8° and a neutralization value of 207.5 is present in the acids from alfalfa seed oil. This corresponds to daturic acid ($C_{17}H_{34}O_2$), obtained by Meyer and Beer[3] from datura oil with a melting point of 59.5° and a neutralization value of 207.6, and is the same as Krafft's synthetic margaric acid. In this connection it is interesting to note that Meyer and Eckert[4] obtained an acid of the same formula as daturic, from coffee-berry oil, having a melting point of 57°, which, when taken in connection with the above results, points to the existence of two metameric forms of daturic acid.

Conclusion.

Alfalfa seed oil is a drying oil whose physical properties resemble safflower oil. It contains practically no volatile acids but 92.5% of the oil is composed of insoluble fatty acids. The fatty acids in the oil contain 90.4% liquid acids and 9.6% solid acids. The liquid acids are composed of oleic, linoleic and linolenic acids, and the solid acids of carnaubic, daturic and possibly behenic acids.

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[1] *Ann.*, **223,** 306 (1884).

[2] *Z. physiol. Chem.*, **64,** 307 (1910).

[3] *Kaiserl. Akad. d. Wissenschaften.* Wien, Jan., 1912.

[4] *Loc. cit.*