ALFALFA SEED OIL

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Abstract

Herein reported are the initial results of an attempt to clarify conflicting statements on the nature and composition of alfalfa seed oil. Its unsaturated acid fraction contains representatives of three common types, linolenic and linoleic acids, neither of which, apparently, is present there in isomeric form, and oleic acid. The study is being continued.

ALFALFA (*Medicago sativa* L.,) called also "lucerne" abroad from the Swiss canton of that name, is one of the oldest known forage crops, but, as age of man's direct and indirect food source is measured, is a comparatively recent arrival on the farms of the United States and Canada. Originally introduced in California over eight decades ago, the cultivation of this hardy perennial, which adapts itself rather well to a great variety of climatic conditions, soon thereafter spread rapidly over the West and then gradually moved eastward until now nearly one-half of the nation's annual crop of alfalfa hay and seed, itself valued at 290 and 6.7 million dollars, respectively, is produced in the central states. That the Western Hemisphere supplies the world's needs of this crop is reflected in the fact that the United States, together with Argentina, produces 98 per cent of it.

Inasmuch as alfalfa is grown primarily because of its excellent fodder value, it is indeed not surprising that investigations centering around it should have included few details concerning the fatty oil of the seed whose production is controlled, more or less, by the demands for means of propagating the plant. In view of this situation, two reasons suggest themselves why an examination of alfalfa seed oil bids fair to be of interest. The first of these is the known existence of at least two conflicting statements in respect to its drying qualities as revealed by iodine number. The second is a report the substance of which challenges attention, for if the findings therein reported are confirmed then in this oil there are not present, as might be expected, two of the most commonly occurring saturated fatty acids, but one whose entity is usually questioned when reported from natural sources; another of the tetracosanic group, designated in this instance as carnaubic; and possibly one other, the next lower homolog of the latter.

Grimm[1] classified this oil with the non-driers because of an observed iodine value of approximately 79. Jacobson and Holmes[2], however, on obtaining a value of almost twice as much, or 154.2, for a Nevada-grown product, quite properly designated it as a drying oil. Since Gardner[3] concurred in this view—a sample obtained by extraction yielded an iodine number of 165—the discrepancy in question can, perhaps, be best explained in the light of any one or more of the reasons usually advanced in similar situations, viz: varietal differences due to soil and climate; state of maturity of the plant when seed was harvested; the time of harvest; the method used in the recovery of the fatty oil and precautions taken, if any, against oxidation during extraction; the portion (liquid or solid fraction) of the oil which had been submitted to analysis.

The relatively low saponification value of 172 which Jacobson and Holmes[2] found doubtless lends some measure of support to their contention that saturated acids above C_{22}, to the exclusion, however, of palmitic and stearic, are present. Identifications, both in respect to them and daturic, whose occurrence in nature seems never to have been proven satisfactorily, were based entirely upon neutralization values and melting points without confirmatory characterization through derivatives. Although offering no quantitative data on the aforementioned acids, these investigators do present two sets thereof pertinent to the three-member unsaturated fraction, deeming those obtained by permanganate oxidation more trustworthy than those resulting from bromide-formation.

With this background as a point of departure, an investigation on alfalfa seed oil, the results of which to date are in part herein presented, was begun not only for reasons suggested in the foregoing summary of the literature but also for the obvious desirability of bringing into play in this case the newer techniques of fatty oil analysis for the purpose of extending the borders of information on the characteristics and composition of this oil whose production, apparently, could be increased many fold without serious detriment to the fodder value of the cropped plant. These constants, together with those of Jacobson and Holmes,[2] which are reproduced here with ready reference and comparison as objective, form the substance of Table I. They were obtained in the analysis of the amber colored oil which had been extracted with petroleum ether in 8.63 per cent yield from about fifty pounds of seed described as "fancy Utah."

TABLE I.
Chemical and Physical Characteristics of Alfalfa Seed Oil.

		Jacobson Holmes
Specific gravity 25°/25°	0.9253	0.9149[1]
Refractive index 20°	1.4797	1.4783[2]
Titer test	19.7	....
Iodine number (Hanus)	167.8	154.2
Saponification number	185.2	172.3
Acetyl value	16.6	19.8
Reichert-Meissl number	0.6	0.4
Polenske number	0.2	....
Soluble acids as butyric (per cent)	1.3	....
Insoluble acids (Hehner number)	92.8	92.5
Saturated fatty acids (per cent)	7.11	9.60
Unsaturated fatty acids (per cent)	89.80	90.4
Iodine number of unsaturated acids	186.48	187.7
Thiocyanogen number of unsaturated acids	103.7	....
Saponification number of unsaturated acids	180.8	....
Unsaponifiable matter (per cent)	3.15	4.40

[1]At 24°/24°. [2]At 17°.

That both low and intermediate molecular weight fatty acids are not present in any noticeable amounts is indicated by insignificant Reichert-Meissl and Polenske numbers. This conclusion seems to be supported by a saponification number which is a bit below the average. Hydroxylated compounds, likewise, are not conspicuous. On the other hand, the thiocyanogen-iodine numbers relationship suggests the presence of representatives of at least three types of unsaturation.

Separation of the unsaturated from the saturated acids was effected by the lead salt-alcohol method of Twitchell as modified by Baughman and Jamieson[4] and identification of the former by means of their bromo addition compounds and their separation by the use, in turn, of diethyl and petroleum ethers.[5] The identity of the components of this fraction was deduced from the following data: (1) linolenic acid from the corresponding, at —10°, ether-insoluble, hexabromo derivative (Br. 63.2 per cent, calculated 63.32 per cent; m.p. 179.3°, theoretical, 177°-181°); (2) linoleic acid from its (hot) petroleum ether-soluble tetrabromo derivative (Br 53.5 per cent, calculated 53.3 per cent; m.p. 113.7°, theoretical 114°); (3) oleic acid, in the residual fraction, through its soluble dibromo derivative (Br 35.85 per cent, calculated 36.18 per cent).

Confirmation of the foregoing identifications was subsequently obtained through the permanganate oxidation products of these acids which were removed from the resulting reaction mixture in the reverse order in which they are herein described. The isolation of linusic acid of melting point 203.5° theoretical 203°-205°) and carboxyl-hydroxyl ratio of 6:1 indicated the presence of a linolenic acid. Since repeated crystallizations did not yield any fraction of lower melting point than that originally found, it was concluded that no isomers of linolenic acid are present. A relatively larger quantity of sativic acid (m.p. 157.6°, COOH/OH = 4), indicative of the presence of a linoleic acid was obtained. In this case, too, replicated crystallizations did not produce an acid of different melting point. It was concluded, therefore, that linoleic acid does not occur in this oil in its two isomeric forms. The presence of oleic acid was confirmed in the formation of dihydroxystearic acid (m.p. 131.3°, theoretical 131°, COOH/OH = 2).

Using iodine and thiocyanogen numbers of the unsaturated acid fraction as a basis of calculation, it appears that this oil contains approximately 1.4 per cent oleic acid, 67.5 per cent linoleic acid and 20.8 per cent linolenic acid. These values are of the general order of magnitude of those which Jacobson and Holmes[2] found by the separation of the permanganate oxidation products of these acids. They are, in the order named, 3.30 per cent, 73.20 per cent and 23.50 per cent.

A report on the nature of the acids comprising the saturated group will be made at some future time after studies now in progress thereon will have been completed.

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ELECTROMETRIC TITRATION OF DICHROMATE GLYCEROL SAMPLES*

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Abstract

For routine control, glycerol is usually determined by oxidation of the sample with potassium dichromate in an excess of sulfuric acid with back titration of the excess dichromate solution after oxidation with ferrous sulfate solution. The usual procedure is to use potassium ferricyanide as an external indicator on a spot plate. The use of an electrometric titration apparatus with platinum and tungsten electrodes as an internal indicator is described. The apparatus is very easily constructed and gives rapid and accurate results with a sharp endpoint under all kinds of lighting conditions by any analyst who can read a buret—even by one who is color blind.

FOR control purposes in soap and glycerol manufacture glycerol is usually determined through the oxidation of a prepared sample by an excess of potassium dichromate in the presence of an excess of sulfuric acid. The most common procedure is to titrate the excess of the dichromate solution, after oxidation is completed, with ferrous sulfate solution using potassium ferricyanide as an external indicator on a spot plate. Randa (5) has recently described a method in which the dichromate solution after oxidation is made to volume and used to titrate standard ferrous sulfate solution, using diphenylamine as internal indicator.

For a number of years in the Procter & Gamble laboratories, the excess dichromate after oxidation has been titrated in the oxidation flask without any transfer or further treatment other than cooling. Ferrous sulfate is used for the titration with an electrometric setup as internal indicator. As far as the authors have been able to learn, the electrometric method has not been used in other laboratories for the titration of the excess dichromate

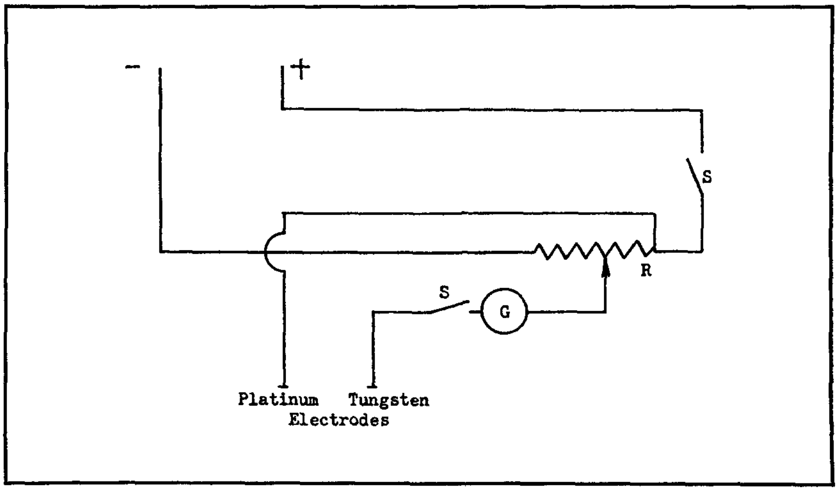

FIGURE 1. WIRING DIAGRAM.
S. Double-pole, single-throw switch.
G. Galvanometer.
R. Potentiometer.
Batteries (4 dry cells in series).

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