The Determination of the Oil Content of Ground Tung Fruit

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THE determination of the oil content of tung fruit based on extraction of the oil from the kernels is expensive due to the high labor costs involved in separating and flaking or grinding the kernels from the large number of fruits required for a proper sample (2, 3). Hence it would seem advantageous to grind and extract the whole fruit.

As the fruit can be readily ground in a Wiley mill, the problem becomes one of obtaining satisfactory oil extraction from the ground material and of obtaining a suitable correction for substances other than tung oil extracted from the ground hull and shell and for the adsorption of true tung oil by the particles of hull. That the problem of finding a satisfactory correction factor is not a simple one is evidenced by the literature, in which several different factors have been recommended (4, 5, 6). The procedure in which whole fruits are ground and extracted has been designated the "whole fruit" method and that in which the kernel only is ground or flaked and extracted, the "component" method. This terminology will be adopted in this report.

In 1947 it was found that the Hamilton-Gilbert procedure (1), in which the extraction is made in a Waring Blendor, could be used for the whole fruit method with no significant loss in precision as compared with the component method. Further study was concentrated on obtaining a close estimate of the accuracy of the whole fruit determinations in terms of the component method, in which the oil is extracted from the kernels, the same portion from which the true commercial tung oil is expressed. In 1947 each of 29 samples of whole tung fruit was divided into two subsamples of 50 fruits each, one of which was analyzed by the whole fruit and one by the component method.

The Hamilton-Gilbert method (1) was used for the component analysis, a 10-gm. sample of the flaked kernel being extracted in a Waring Blendor with hexane, and the extract cleared by settling. A representative sample of the kernels from air-dried fruits is flaked. The flaked kernel material is thoroughly mixed, a 10-gm. portion of the sample is transferred to a Waring Blendor, 170 ml. of Skellysolve B are added, and the material is agitated for 5 minutes. The blendor disintegrates the flaked kernel and the oil is dissolved in the solvent. The resulting solution of oil, with its suspension of tung meal, is transferred through a funnel into a 250-ml. volumetric flask, pref-

erably a flask that is calibrated from 245 to 255 ml. in 0.5-ml. divisions. The entire contents of the blendor jar are carefully rinsed into the flask, enough solvent is added to adjust the total volume to about 252 ml., the contents are thoroughly mixed, and the flask is set aside to cool and settle at room temperature.

In determinations on kernels from air-dried fruits the solutions are usually sufficiently clear after settling for about an hour. Samples extremely low in moisture content appear to require a longer time to settle. The solution comes from the blendor at a temperature of 40° to 50°C. and hence will shrink several milliliters on cooling to room temperature. Following settling, the final volume of the contents is read from the calibrations of the flask, and a 50-ml. portion is pipetted into a thoroughly cleaned, tared 250-ml. beaker.

For routine determinations of a number of samples, only one graduated flask is required for each group of samples. Ordinary 250-ml. volumetric flasks are used for the rest of the group, all being made to volume at the same temperature in a water bath. Subsequent volume changes during the settling are corrected for by reading the graduated flask. A 1-ml. Mohr pipet can be used in calibrating the flasks above and below the 250-ml. mark. For precise work the temperature of the oil solution should be within 1° of room temperature at the time of aliquoting.

The solvent is evaporated on a steam bath, and the final traces are removed in a vacuum oven at 70°C. and 4-mm. pressure.

The beaker plus oil is weighed after cooling to room temperature. Exposure to the air in the balance room does not materially affect the weight. The weight of the oil in the beaker (obtained by difference) multiplied by one-fiftieth of the volume of the contents of the flask at the time the aliquot is taken gives the weight of oil in the sample used.

This method was found (1) to be superior in accuracy and precision to the Goldfisch extraction of flaked kernels, which had been previously found (3) to compare favorably with the Butt tube procedure.

The same procedure was followed in the whole fruit analyses excepting that a 10-gm. sample was used of fruit ground to 2-mm. in a Wiley mill. In addition, a modification of the original method (1) was used in which the extract was filtered by suction through a 9-cm. B & A No. 0 filter paper on a Buchner funnel covered with 10 g. of Dicalite 4200 or Hyflo Super Cel filter aid dispersed in 25 cc. hexane and packed tight by suction. A 10-g. portion of extracted ground tung fruit was also effective as a filter aid.

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The filtrate was caught in a 500-cc. suction flask and transferred to a 250-ml. volumetric flask. The filter pad was washed with 3 x 25 ml. of hexane, and the washings added to the volumetric flask, which was then made to volume. An aliquot was then taken for oil as in (1). No correction was made for unextracted oil in the residue and filter aid.

Both whole fruit determinations had a satisfactory degree of precision, and a high degree of correlation was shown to exist between the determinations of oil content obtained by the whole fruit method and those obtained by the component method (Table I). The average of 29 whole fruit determinations,

TABLE I

Determinations, by Three Different Methods of the Percentage Content of Oil of Air-dry Tung Fruits

Trees from which Samples Were Taken	Component, Extract Cleared by Gravity	Whole Fruit, Extract Cleared by Gravity	Whole Fruit, Extract Cleared by Filtration
	%	%	%
F-3	23.2	23.5	22.3
F-4	22.0	22.2	21.1
F-11	25.0	26.4	25.1
F-18	23.0	23.8	23.0
F-19	19.1	20.1	19.2
F-24	22.1	23.6	22.2
F-28	22.1	21.9	21.2
F-30	23.2	23.1	22.1
F-40	22.9	24.9	23.0
F-42	20.4	21.2	20.6
F-56	21.7	22.0	20.8
F-58	15.5	16.2	15.1
F-59	24.9	25.2	22.6
F-62	22.3	23.5	22.9
F-75	24.6	26.4	24.6
F-76	20.5	23.8	23.0
F-99	22.8	24.9	22.2
F-138	16.0	15.6	14.3
F-158	16.8	17.7	16.7
F-206	19.0	21.1	19.5
F-217	22.8	23.3	22.4
F-229	21.2	22.3	21.1
F-303	19.0	19.7	18.8
F-305	24.8	26.4	23.9
R-1	19.9	20.3	18.6
R-2	20.8	21.2	19.9
R-3	23.5	24.7	22.2
R-4	20.3	20.8	18.7
R-6	22.0	22.2	21.4
Average	21.43	22.34	20.98

with gravity separation, was 22.34%, 0.91 percentage unit higher than the average by the component method. Inasmuch as a high degree of correlation exists between results by the component and by the whole fruit methods, the relation between the two is best expressed by a regression equation (7). The equation for the straight-line relationship between determinations by the whole fruit gravity separation method and those by the component method is:

Estimated component value = 0.8653 (value by whole fruit method) + 2.10.[2] There was considerable variation however in the readings for component and whole fruit values on the same sample of fruit. This was believed to result from the use of subsamples of only 50 fruit (2, 3).

The average of the whole fruit determinations in which the extract was filtered was 20.98%, 0.45 percentage unit lower than the average of the component analyses. As has previously been explained (1), in gravity separation the volume of the residue is included in the total volume of the solution; and if the concentration of unextracted oil in the residue is about equal to that in the supernatant solution, little error is introduced. In the filtration method how-

[2]Additional statistics on this and the other equations in this paper are given in Table IV.

ever any oil remaining in the residue or adsorbed on the filter paper represents a loss. The equation relating the component and the whole fruit filtration methods is:

Estimated component value = 0.9105 (value by whole fruit method) + 2.33.

The equations have the same general form, which indicates that the relationship between the component and the whole fruit methods is the same in both cases although, for the reason stated above, a plus correction is required over the range of 18% to 25% oil in the whole fruit when the filtration procedure is used and a minus correction when gravity separation is used. The two methods differ only slightly in degree of precision. The gravity separation will generally be preferred for multiple determinations because less labor is required per determination, but separation by filtration permits completing a single determination in a shorter time.

In 1948, 200 fruits taken from each of 34 individual trees were first divided into two samples of 100 fruits each. Each 100-fruit sample was then divided into two subsamples of 50 fruits each, one of which was analyzed by the component and one by the whole fruit method. The average of the whole fruit determinations was 22.62%, 1.00 percentage unit higher than the average of the component values (Table II),

TABLE II

Determinations of Percentage of Oil in Air-dry Tung Fruits

Trees from which Samples Were Taken	Component Method Average	Whole Fruit Method Average
	%	%
F-5	21.6	21.5
F-17	21.3	21.4
F-18	21.5	21.3
F-19	19.4	20.7
F-20	17.6	19.6
F-21	22.7	23.4
F-22	21.2	23.0
F-23	18.6	20.0
F-24	20.0	21.1
F-30	19.4	21.7
F-36	19.7	21.3
F-39	20.0	20.7
F-42	21.5	22.4
F-46	22.2	22.8
F-75	24.5	26.1
F-76	22.1	23.4
F-79	22.6	23.3
F-81	25.5	26.7
F-84	19.4	21.3
F-85	19.6	19.6
F-86	22.4	24.0
F-96	22.1	22.7
F-111	21.6	22.2
F-114	23.7	25.3
F-116	23.0	23.8
F-173	24.5	26.0
F-174	23.5	24.6
F-175	22.3	23.3
F-187	22.0	22.5
F-191	18.2	19.3
F-237	19.0	19.6
F-260	23.9	24.8
F-263	23.3	24.3
F-264	25.2	25.5
Average	21.62	22.62

and an equation for the relationship between component and whole fruit determinations was found to be of the same general form as that obtained in 1947, namely:

Estimated component value = 0.9196 (value by whole fruit method) + 0.82.

The gravity separation data for 1947 and 1948 were combined and from the total of 97 samples a more reliable equation was obtained. The average of the

component values was 21.53%, 0.96 percentage unit lower than the average of the whole fruit determinations. The equation calculated from the 97 pairs of readings is:

Estimated component value = 0.8958 (value of whole fruit method) + 1.37.

This equation means that if for a given sample a reading of 25.00 percentage units of oil is obtained by the whole fruit method, a reading of 23.77 percentage units would be obtained by the component method, a difference of 1.23 percentage units of oil. A sample analyzing 18.00 percentage units of oil by the whole fruit method would give 17.49 units of oil by the component method, a difference of only 0.51 unit. Thus, the use of an average difference of —0.96 percentage unit over the whole range is not justified.

It is evident however that if the correction factor were related only to the contamination of the true kernel oil with substances extracted from the hull, a sample in which the ratio of kernel to hull is low, as is generally the case when oil content of the whole fruit is low, would require the greater correction. The regression coefficient would then have a value greater than unity. The regression coefficients actually found were always less than unity.

An experiment was designed to determine more precisely the effect of varying ratios of kernel to hull and shell on oil extracted and the correction required. Mixtures of ground hull and shell with ground whole fruit were prepared in which the percentage of kernel ranged from 32.2 to 0.0. In such mixtures of practically homogeneous ground materials sampling error is almost entirely eliminated. The standard of comparison was the oil content of the mixture calculated from its know kernel content and the percentage of oil in the kernel as previously determined. The data are given in Table III and fit the regression equation:

Calculated oil content = 0.9804 (value determined) — 0.78.

The fiducial limits for the regression coefficient at the .05 level of probability are 1.0008 and 0.9600. Thus it is probable that, even in this wide range of mixtures, the true value of the coefficient is less than unity. On the assumption that the correction factor merely compensates for substances other than true tung oil extracted from the hull and shell, a relatively high correction would be expected for those mixtures consisting largely of hull and shell with very little kernel. Other factors, such as adsorption of kernel oil by the hull or decreased efficiency of extraction of oil in the presence of large amounts of hull and shell, must play an important part.

The actual samples of fruit used in arriving at the combined regression equation undoubtedly varied not

TABLE III
Percentage of Oil in Artificial Mixtures of Ground Tung Kernel with Ground Hull and Shell

Kernel in Mixture	Oil by Extraction	Oil as Calculated
%	%	%
32.2	21.4	20.3
30.6	20.2	19.3
29.0	19.4	18.3
27.4	18.7	17.3
25.8	17.5	16.2
24.2	16.5	15.2
22.6	14.8	14.2
20.9	14.5	13.2
19.3	13.1	12.2
17.7	12.6	11.2
16.1	11.5	10.2
14.5	9.7	9.1
12.9	9.1	8.1
11.3	8.0	7.1
9.7	7.3	6.1
8.1	5.8	5.1
6.5	4.4	4.1
4.8	4.1	3.0
3.2	2.7	2.0
1.6	2.1	1.0
0.0	1.0	0.0

only in ratio of kernel to hull and shell but also in other respects. It seems logical to expect that nutritional conditions that affect filling of the nuts with kernel would also affect the storage of substances other than oil in the hulls. Hence a close agreement between the natural and the artificially prepared samples would not be expected.

The whole fruit method must be considered empirical and therefore valid only over a calibrated range. It is believed that the best method of relating results of analyses by two empirical chemical procedures is by a regression equation and that such regression equations are of very general application.

The data reported here were obtained with tung fruit samples representative of those ordinarily analyzed in the laboratory of the Bureau of Plant Industry, Soils, and Agricultural Engineering. They were uniformly dry (7-13% moisture), and percentage of oil in the whole fruit ranged from 16.0 to 25.0. They are not representative of samples of tung fruit analyzed as a basis for commercial transactions. Such commercial samples vary widely in moisture content, and percentage of oil in the whole fruit usually ranges from 10.0 to 20.0; commercial lots of tung fruit with average oil content as high as 25% are seldom delivered to the mills. Before this method can be recommended for making analyses to be used as a basis for commercial transactions, further work must be carried out on samples representative in both oil and moisture content of fruit customarily delivered to the processing plants.

Summary

The Hamilton-Gilbert method for oil determination has been adapted to the method of grinding and extracting whole tung fruit. A sample of 100 fruit is ground in a Wiley mill with sharp blades. A 10-gm.

TABLE IV
Statistics Pertaining to Calculation and Use of Regression Equations

Data Correlated	Correlation Coefficient (γ)	Regression Coefficient (β)	Standard Error of Regression Coefficients (Sβ)	Standard Error of Estimate (Sy.x)	Predictive Value (γ²×100)
Table I					%
Whole fruit cleared by gravity with component	.955	.8653	.0518	.76	91.2
Whole fruit cleared by filtration with component	.937	.9105	.0655	.89	87.6
Table II					
Whole fruit cleared by gravity with component	.922	.9196	.0474	.81	85.0
Tables I and II combined					
Whole fruit cleared by gravity with component	.934	.8958	.0350	.79	87.2
Table III					
Oil content by extraction with calculated oil content	.999	.9804	.0098	.28	99.8

portion is then extracted in a Waring Blendor and oil determined by the procedure previously described or a modification herein described. To approximate closely the values that would be obtained by the strictly accurate but more laborious method of separating kernels from hull and shell and extracting the kernels only (component method), a correction is required for non-oil substances extracted from the hull and for factors such as adsorption of the kernel oil on particles of hull and decreased efficiency of extraction of the kernel oil in the presence of ground hull and shell. This correction factor is not constant throughout the whole range in oil content found in whole tung fruits, and the best estimate of true tung oil content is obtained by the formula:

Estimated component value = 0.8958 (value by whole fruit method) + 1.37.

The use of a regression equation such as that above in relating results of two empirical chemical methods

is believed to be of very general application. The specific formula here arrived at was determined from 97 samples of air dry fruit ranging in oil content from 16.0 to 25.0%. It is not recommended for use with commercial samples of tung fruit, which vary widely in moisture content and usually range from 10.0 to 20.0% in oil content until adequate tests with such samples have been made.

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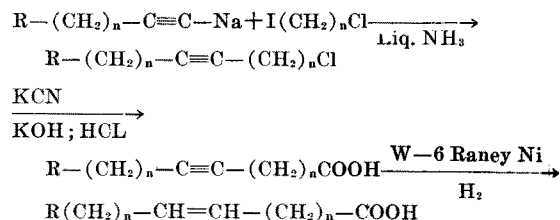
Syntheses of Cis- and Trans-7- and 8-Octadecenoic Acids: Comparison of the Properties of Cis- and Trans-6-, 7-, 8-, 9-, and 11-Octadecenoic Acids

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AS a result of extensive investigations on both animal and seed fats Millican and Brown (1) postulated the natural occurrence of appreciable quantities of isomeric octadecenoic acids occurring along with oleic acid. However they did not isolate these isomeric acids for lack of methods for effecting the necessary separations. In keeping with one of the principal research objectives of this laboratory, that of developing methods for the isolation and purification of naturally occurring fatty acids, it was desired to prepare pure octadecenoic acids with the double bond in various positions near that found in oleic acid so that solubilities of these acids could be determined and methods for separation worked out.

In order to initiate this program it became necessary to prepare pure samples of iso-oleic acids by methods which would lead unequivocally to pure products. Since it seemed more likely that the most probable naturally occurring iso-oleic acids would be those with the double bond close to the 9-position, we decided to synthesize the cis-7-, 8-, and 11-octadecenoic acids. In addition, pure cis-6-(petroselenic) and cis-9-(oleic) octadecenoic acids were prepared by a combination of fractional distillation and low temperature crystallization. Isomerization of all of these cis acids with selenium, followed by low temperature crystallization, gave us the pure trans acids. Thus five cis-trans pairs of octadecenoic acids were available for various types of study, including solubility and infra-red examination. The infra-red studies were particularly significant in view of the recent reports by Swern, Heether, et al. (14).

Our syntheses were patterned after the work of Ahmad and Strong (2) on cis- and trans-11-octadecenoic acids. Our results with these acids checked theirs very closely and hence will not be described here. Our synthesis of the 7- and 8- acids followed their basic pattern with important modifications. Their method (2) involved the condensation of an alkynyl sodium derivative with a chloro-iodoalkane to give a 1-chloroalkyne which could then be converted to an alkynoic acid and thence to an alkenoic acid.

$$R-(CH_2)_n-C\equiv C-Na+I(CH_2)_nCl \xrightarrow{\text{Liq. } NH_3}$$
$$R-(CH_2)_n-C\equiv C-(CH_2)_nCl$$

$$\xrightarrow[\text{KOH; HCL}]{KCN}$$

$$R-(CH_2)_n-C\equiv C-(CH_2)_nCOOH \xrightarrow[H_2]{W-6 \text{ Raney Ni}}$$
$$R(CH_2)_n-CH=CH-(CH_2)_n-COOH$$

We found that bromo-chloroalkanes are superior to the iodo-chloroalkanes when the alkyl carbon chain is C_6 or less. If $I(CH_2)_4Cl$ or $I(CH_2)_5Cl$ or the respective bromo-chloroalkanes are desired, then tetrahydrofuran and tetrahydropyran are much better starting materials for these syntheses than are either $HO(CH_2)_4OH$ or $HO(CH_2)_5OH$, the compounds which would be called for if the original method (2) of preparation is followed.

Experimental

Cis- and Trans-7-Octadecenoic Acids

ω-Chloro-amyl Acetate. ω-Chloro-amyl acetate was prepared according to Synerholm (3), by treating 734 g. of tetrahydropyran with 735 g. of acetyl chlo-