THE DEVELOPMENT OF A SHORT COMMERCIAL METHOD FOR THE DETERMINATION OF OIL IN CEREALS; A COMPARISON OF THE TIME REQUIRED FOR THE COMPLETE EXTRACTION OF WHEAT OIL FROM THE WHEAT GERM WITH THAT REQUIRED RESPECTIVELY BY SULPHURIC ETHER AND PETROLEUM ETHER.

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA BY LILLIAN L. NYE, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ARTS.

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Purpose: The purpose of this thesis was to work out a short commercial method for determining the oil in wheat, thereby reducing to a minimum the length of time required which is sixteen to twenty-four hours; to compare the relative amount of oil extracted respectively in varying durations of time by the solvents sulphuric ether and petroleum ether; and to determine the length of time required by each solvent for a complete extraction.

Historical Resumé: The common solvents[1] are sulphuric ether[2], petroleum ether[3], alcohol, carbon bisulphide, chloroform, acetone, and carbon tetrachloride, but sulphuric ether and petroleum ether are most generally accepted.

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1) Jour.of the Amer.Chem.Soc., Vol. 29, P.766.
2) Amer.Chem.Jour., Vol. 13, P. 13.
3) Jour. of the Amer. Chem. Soc., Vol.20, P.948.

The time required for a complete extraction varies with different solvents, usually sixteen to twenty-four hours.

The forms of apparatus are Knorr's[1] Soxhlet's, Wiley's and modified forms.

A.P. Bryant[2] has compared the percentages of fat obtained from substances of vegetable origin by means of different solvents and has obtained a rapid method for determining fat by means of carbon tetrachloride. His carbon tetrachloride method(two hours) gave concordant results with carbon bisulphide(four hours) and ether(sixteen hours) extractions. There is one objection to his method, that is, the difficulty of driving off the last traces of carbon tetrachloride. This difficulty is obviated by this paper.

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1) Bulletin 28, U.S.Dept. of Agric.(1890) P. 96.
2) Jour. of Amer.Chem. Soc. Vol. 26, P. 568.

Dr. G.B. Frankforter and Dr. E.P. Harding[1] have made a complete study of wheat oil.

Apparatus: The apparatus used for the petroleum and sulphuric ether extractions was a reflux Liebig condenser, a Soxhlet extraction apparatus, Schleicher and Schüll capsule and two hundred cubic centimeter Erlenmeyer flasks and a water bath.

The apparatus used for the modified method was a reflux Liebig condenser; a one hundred and fifty cubic centimeter straight tube; a water bath; a blow-off tube resembling Werner Schmidt's[2] for the determination of fat; one hundred cubic centimeter Erlenmeyer flasks and a centrifuge.

Solvents: Carbon Tetrachloride.- The carbon tetrachloride was purified by collecting portion which

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1) Jour. of the Amer. Chem. Soc., Vol. 21, P. 758.
2) Leach's Food Inspection and Analysis.

distilled over at temperature of boiling water. Boiling point was 76°C.

Sulphuric Ether.- The sulphuric ether was washed free from alcohol with water and the water removed with calcium chloride. After standing over night it was re-distilled with calcium chloride and that portion collected with a boiling point of 34°C.

Petroleum Ether.- The petroleum ether was purified by re-distillation on water bath, collecting portion which distilled between 55°-68°C.

Methods and Manipulations:

Preparation of Sample.- The wheat germ was the germ obtained at the Washburn Crosby mill by crushing wheat kernel and separating germ from rest of wheat by bolting. The sample was hand-picked to separate larger impurities and then put through an eighty mesh sieve to separate germ from starch and impurities.

Drying of Sample.- Weighed out a one gram sample into a flat bottomed porcelain dish and heated to constant weight in an oven provided with a thermostat at a temperature of 99°-101°C. Required about two hours. For need of special precautions in drying see article by Sherman Leavitt.[1]

For use in extractions, larger samples of twenty to thirty grams were weighed out and dried to slight increase in weight.

Extraction of Oil.- For petroleum and sulphuric ether extractions, weighed out five gram samples into a Schleicher and Schüll capsule and placed capsule in Soxhlet, fitted Soxhlet to reflux condenser and Erlenmeyer flask by means of corks so apparatus was air-tight. Clamped apparatus in a vertical position to ringstand on a water bath. Poured in ether from top

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1) The Jour. of Indus. and Eng. Chem., Vol. 2, P.19, Jan, 1910.

carefully so as not to loosen sample from cup. Poured in enough ether to fill Soxhlet twice. Heated bath to boiling and extracted at rate of eight to ten siphonings per hour. Continued the extraction of five gram samples for three consecutive periods of five hours each. Extracted two gram samples with petroleum ether and sulphuric ether for ten and sixteen hours respectively.

After extraction, the flask was disconnected from Soxhlet and extract carefully filtered through a 7 cm., 589 white band filter paper into a 100 cc. Erlenmeyer flask which had previously been thoroughly cleaned, dried and weighed to constant weight. Washed extraction flask and filter with successive small portions of solvent to completely remove all extract. Distilled off solvent using a modified Soxhlet and reserved solvent for second use. Brought oil to constant weight by immersing flask in boiling water in a casserole. Removed flask from casserole,

cooled by allowing the tap water to flow over it, wiped dry and quickly weighed. Repeated to constant weight or a slight increase in weight. Required about forty-five minutes to bring to constant weight.

The first two or three siphonings with sulphuric ether were of a deeper yellow than were those with petroleum ether. The color disappeared after four or five siphonings. The oil on first extraction is a golden yellow, but on drying becomes slightly darker or of a reddish-brown color. The sulphuric ether extracted something which did not give a clear oil and which was insoluble in ether. This method requires about twenty hours for completion, including the time required for the drying of the sample and oil.

Modified Method.- The sample was dried as above. An air-dried sample may be used. A two gram sample was weighed into the extraction tube, added exactly twenty-five cubic centimeters of carbon tetrachloride, connected tube with reflux condenser; placed tube in water bath. Extracted one hour at temperature of boiling

water. Removed tube and distilled off exactly twelve and one half cubic centimeters of carbon tetrachloride. Added twenty five cubic centimeters of absolute alcohol mixed thoroughly by gentle shaking in hand. Added twelve and one half cubic centimeters of distilled water. Placed rubber stopper previously moistened with water into tube and shook well. Added forty-five cubic centimeters of petroleum ether and shook thoroughly. Placed in centrifuge and whirled ten minutes, at first slowly and then increased to high speed. Removed and allowed bubbles to break. Had a clear separation of two layers. Removed rubber stopper and inserted blow-off tube. Blew off ether layer, after making sure there were no bubbles in tube, through 589, 7 cm. white band filter paper into tared flask previously cleaned as above. If bubbles were present drew back into tube and allowed bubbles to settle completely. Presence of one bubble of lower layer leads to serious error in percentage of oil. Washed with three successive portions of

petroleum ether and blew off into flask through filter paper. Washed filter thoroughly free from any adhering oil. Labelled flask, 1st ''blow off''.

To residue in tube added five cubic centimeters of carbon tetrachloride and five cubic centimeters of absolute alcohol and mixed well.Added forty cubic centimeters of petroleum ether and inserted rubber stopper,previously moistened with water, into tube and shook thoroughly. Whirled in centrifuge five minutes. Removed and allowed bubbles to break. Clear separation. Then removed stopper and inserted blow-off apparatus and blew off ether layer through same filter as before into a second tared flask. Washed with three successive portions of ten cubic centimeters of petroleum ether and added washings to flask. Used same precautions as above. Labelled flasks 2nd ''blow-off''.

To residue in tube added five cubic centimeters pf carbon tetrachloride, five cubic centimeters of absolute alcohol and forty cubic centimeters of petroleum ether

and proceeded as in second portion above. Repeated until had four ''blow-offs''.

Then distilled off ether with modified Soxhlet. Dried oil by immersing flask in boiling water in casserole as above. Cooled flask under tap and wiped dry and weighed quickly to constant weight. To bring to constant weight, made weighings after thirty minutes and after successive fifteen minute intervals until had an increase in weight. Took last reading before increase in final weighing. Less time was required to bring last three ''blow-offs'' to constant weight.

Too great care can not be exercised in the manipulation as one bubble causes serious error. As a precaution always observe closely whether or not there are any bubbles in the tube and if so, draw back and allow to subside.

As a preliminary method the extraction was made for one hour as above and after adding the absolute alcohol and water and ether, the flask was allowed to stand from

six to twelve hours but it was found that the layers would not separate completely.

The centrifuge was then introduced as a means for separation. Difficulties arose in separation constantly until the above portions of solvents were used. The methods first employed differed in not using five cubic centimeters of carbon tetrachloride and five cubic centimeters of absolute alcohol but one cubic centimeter of carbon tetrachloride or none at all. It was further found that a separation was impossible unless the exact amount, twelve and one half cubic centimeters, of carbon tetrachloride and above amounts of absolute alcohol, water and petroleum ether were present. Ninety-five per cent alcohol and ether of low boiling point 40°-50°C caused reverse incomplete separation.

The object in making the separate blow-offs was to find out how many were necessary to remove all of the oil. For a complete separation three are sufficient

for removal of wheat oil. The fineness of the substance gives a larger percentage of oil in the first blow-off, but three blow-offs are necessary.

The oil obtained by this method is a lighter yellow and seemingly purer. On drying the oil it does not discolor as above. It contains a very much smaller amount of insoluble matter than does sulphuric ether. The time required for a complete determination of oil by this method is four and one half hours.

Tabulation of Data:

Determination of Moisture in Wheat Germ.-

1 gram samples.

(1) Weight of dish and germ
before drying----------15.3330 grms.
Weight of dish and germ
after drying----------15.2466 ''
Weight of moisture-------- 0.0864 grms.--8.64 o/o

(2) Weight of dish and germ
before drying----------15.3960 grms.
Weight of dish and germ
after drying----------15.3088 ''
Weight of moisture-------- 0.0872 grms.--8.72 o/o

Average percentage of moisture----------8.68 o/o

Extractions with Petroleum Ether.

Dry Sample.

(1) Ten hour extraction of two five hour periods.

Percentage of moisture----------8.5 o/o

1st five hours.

Weight of sample---5.0000 grms.

Weight of oil---------- 0.5376 grms.----10.752 ''

2nd five hours.

Weight of oil---------- 0.0026 '' ---- 0.052 ''

Total Percentage of oil----------10.804 o/o

(2) Ten hour extraction of two five hour periods.

Percentage of moisture---------- 8.5 o/o

1st five hours.

Weight of sample---5.0000 grms.

Weight of oil---------- 0.5390 grms.----10.78 o/o

2nd five hours.

Weight of oil---------- 0.0076 '' ----- .152 ''

Total percentage of oil----------10.932 o/o

(3) Ten hour extraction of two five hour periods.

Percentage of moisture----------8.5 o/o

First five hours.

Weight of sample---5.0000 grms.

Weight of oil----------0.5368 grms.-----10.736''

2nd five hours.

Weight of oil----------0.0102 ''------- 0.204 ''

Total percentage of oil---------- 10.940 o/o

(4) Fifteen hour extraction of three five hour periods.

Percentage of moisture---------- 8.5 o/o.

1st five hours.

Weight of sample---5.0000 grms.

Weight of oil---------- 0.5368 grms.----- 10.736''

2nd five hours.

Weight of oil---------- 0.0060 ''------- 0.120''

3rd five hours.

Weight of oil---------- 0.0036 ''------- 0.072 ''

Total percentage of oil---------- 10.928 o/o.

Average of above four extractions---------- 10.901 ''

PETROLEUM ETHER.

Six hour extraction. Dry Sample.

Percentage of moisture----------8.02 o/o

Weight of sample---5.0000 grms.

(1) Weight of oil-------0.5460 grms.----10.92 o/o

(2) Weight of oil-------0.5506 '' -----11.012 ''

Average percentage for six hour extraction----10.966 o/o

Ten hour extraction. Dry Sanple.

Percentage of moisture----------8.3 o/o

Weight of sample---2.0095 grms.

(1) Weight of oil-------0.21886 grms.--10.889''

(2) Weight of sample-2.0055 grms.

Weight of oil-------0.22200 ''-----11.069''

(3) Weight of sample-2.0087 grms.

Weight of oil-------0.22014 ''-----10.959''

(4) Weight of sample-2.0039 grms.

Weight of oil------ 0.22260 ''-----11.108''

Average percentage for ten hour extraction--- 11.006 o/o.

Sixteen hour extraction. Dry Sample.

Percentage of moisture---------- 8.3 o/o

(1) Weight of sample---2.0049 grms.

Weight of oil----------0.2206 grms.-11.003 o/o.

(2) Weight of sample---2.0035 grms.

Weight of oil----------0.22112 ''---11.036 ''

(3) Weight of sample---2.0031 grms.

Weight of oil----------0.22054 ''---11.009 ''

(4) Weight of sample---2.00450 grms.

Weight of oil----------0.22196''--- 11.073 ''

Average percentage for sixteen hour extraction--11.030 o/o.

SULPHURIC ETHER.

Dry Sample.

(1) Ten hour extraction of two five hour periods.

Percentage of moisture----------8.3 o/o

1st five hours.

Weight of sample---5.0000 grms.

Weight of oil---------- 0.5784 grms.--11.568 ''

2nd five hours.

Weight of oil---------- 0.0082 ''----- .164 ''

Total percentage of oil----------11.732 o/o

Sulphuric Ether.

Ten hour extraction.

Dry Sample.

Percentage of moisture----------8.3 o/o

(1) Weight of sample---2.0033 grms.
Weight of oil----------0.23374grms.-11.667 o/o

(2) Weight of sample---2.0097 grms.
Weight of oil----------0.23032 ''-- 11.46 ''

(3) Weight of sample---2.0055 grms.
Weight of oil----------0.2296 ''--- 11.448 ''

(4) Weight of sample---2.0045 grms.

Weight of oil----------0.22564 grms.---11.257 o/o

(5) Weight of sample---2.0029 grms.

Weight of oil----------0.22724 ''------11.345 ''

Average percentage of oil of ten hour extractions.-11.567 o/o

Sulphuric Ether.

Sixteen hour extraction.

Dry Sample.

Percentage of moisture---------- 8.3 o/o

(1) Weight of sample----2.0025 grms.

Weight of oil----------0.23362 grms.-11.666 o/o

(2) Weight of sample---2.0027 grms.

Weight of oil----------0.2300 ''----11.484 ''

(3) Weight of sample---2.0017 grms.

Weight of oil----------0.22962 ''----11.471 ''

(4) Weight of sample---2.0023 grms.

Weight of oil----------0.2324 grms.---11.606 ''

Average percentage for sixteen hour extractions--11.557 o/o.

A glance at the above data as per the following table,

Solvent	o/o of oil extracted in 10 hrs. af 5 hr. periods.	o/o of oil extracted in 10 hrs.	o/o of oil extracted in 16 hrs.
Petroleum Ether	10.901	11.006	11.030
Sulphuric Ether	11.732	11.567	11.557

revealed some discrepancies relative to the use of sulphuric ether as a solvent. The amount of extract in each case by sulphuric ether was considerably higher than that with petroleum ether, which verified Maxwell's statement[1], that something besides oil was extracted by the ether.

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1) Amer. Chem. Jour., Vol. 13, P. 13.

Furthermore, an increase in the period of extraction with petroleum ether as a solvent increased the amount of extract but did not necessarily cause an increase in fatty bodies only, according to Maxwell. There should have been a corresponding increase in amount of extract with sulphuric ether as a solvent, but owing to the very great difficulty of obtaining uniform amounts with this solvent, the reverse condition resulted. The petroleum ether appeared to give a purer oil as evidenced by color and less turbidity on standing. However, the ether extract did not represent a pure oil but all other bodies soluble in ether.[1]

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1) Jour. of the Amer.Chem. Soc. Vol. 20, P. 304.

Modified Method.

Dry Sample.

Percentage of moisture----------8.2 o/o

(1) Weight of sample---2.0067 grms.

(a) 1st. blow-off.

Weight of oil----------0.2036 grms.--10.146 o/o

(b) 2nd blow-off.

Weight of oil----------0.0102 ''---- .508 o/o

(c) 3rd blow-off.

Weight of oil----------0.0030 ''----- .149 ''

(d) 4th blow-off.

Weight of oil----------0.00038''----- .019 ''

Total percentage of oil----------10.822 o/o

(2) Weight of sample---2.0017 grms.

(a) 1st blow-off.

Weight of oil----------0.20322 grms.---10.152 o/o

(b) 2nd blow-off.

Weight of oil---0.0092 grms.---------- .459 ''

(c) 3rd blow-off:

Weight of oil---------0.0020 grms.---------.099 o/o

(d) 4th blow-off.

Weight of oil---------0.0006 '' ---------.029 ''

Total percentage of oil----------10.739 o/o

(3) Weight of sample---2.0037 grms.

(a) 1st blow-off.

Weight of oil----------0.2002 grms.------- 9.991 o/o

(b) 2nd blow-off.

Weight of oil---------- 0.0098 '' -------- .489 ''

(c) 3rd blow-off.

Weight of oil---------- 0.0018 '' -------- .089 ''

(d) 4th blow-off.

Weight of oil---------- 0.0006 '' -------- .029 ''

Total Percentage of oil----------10.598 o/o

(4) Weight of sample---2.0049 grms.

(a) 1st blow-off.

Weight of oil---------- 0.2032 grms.-----10.135 o/o

(b) 2nd blow-off.

(b) 2nd blow-off.

Weight of oil----------0.0108 grms.---.538 o/o

(c) 3rd blow-off.

Weight of oil----------0.0034 '' ----.169 ''

(d) 4th blow-off.

Weight of oil---------- 0.0003 ''-----.015 ''

Total Percentage of oil----------10.857 o/o.

(5) Weight of sample---2.0017 grms.

(a) 1st blow-off.

Weight of oil----------0.1998 grms.------9.982 o/o

(b) 2nd blow-off.

Weight of oil----------0.0104 '' ------ .519 ''

(c) 3rd blow-off.

Weight of oil----------0.0030 '' ------ .150 ''

(d) 4th blow-off.

Weight of oil----------0.0004 '' ------ .019 ''

Total Percentage of oil----------10.670 o/o

Average of above five determinations----------10.757 ''

The following table served to elucidate the development of the modified method:

o/o oil first Blow-off	o/o oil second Blow-off	o/o oil third Blow-off	o/o oil fourth Blow-off	Total
(1)10.050	.315	.139		10.504
(2) 9.872	.388	.133		10.393
(3)10.113	.558	.182		10.583
(4) 9.845	.464	.181		10.490
(5)10.324	.509	.131	.148	11.112
(6)10.074	.628	.179	.219	11.10
(7)10.081	.503	.131	.022	10.737
(8)10.471	.449	.109	.009	11.029

(1)-(4) gave fairly concordant totals but the large percentage of the third blow-off necessitated the addition of some solvent to increase the amount of oil in the preceeding blow-offs. Before such a solvent was added, a fourth blow-off was made with the fifth sample, in order to ascertain if approximately all the oil could not be removed in the first three blow-offs. The fourth blow-off proved to be on a par with the third. Accordingly in (6)

one half a cubic centimeter of carbon tetrachloride was added to the residue after the first blow-off and the process carried on from that point as in (1)-(5). It was found that with one trial the fourth blow-off was equally as large as the third. The addition of five cubic centimeters of carbon tetrachloride and five cubic centimeters of absolute alcohol established the method. (7) represented the averages of the four blow-offs of five different determinations run according to the established method. (8) was run to determine whether or not the physical state of the sample had a material effect upon the amount of oil extracted. The germ was pulverized before drying to constant weight. There was an increase in the amount of oil extracted in the first two blow-offs and a corresponding decrease in the last two, but there was no particular gain in that three blow-offs were necessary to remove all but an hundredth per cent of the oil. This basis was made on one run only as time did not permit an extensive investigation.

SUMMARY:

PETROLEUM ETHER.

10-15 hour extractions of two and three 5 hour periods respectively.

o/o of oil extracted in 1st 5 hours	o/o of oil extracted in 2nd 5 hours.	o/o of oil extracted in 3rd 5 hours.	Total o/o
(1)10.752	.052		10.804
(2)10.780	.152		10.932
(3)10.736	.204		10.940
(4)10.736	.120	.072	10.928
Av.10.751	.132		10.901

o/o of oil 6 hour extractions	o/o of oil 10 hour extractions	o/o of oil 16 hour extractions.
(1)10.92	(1)10.889	(1) 11.003
(2)11.012	(2)11.069	(2) 11.036
Av. 10.966	(3)10.959	(3) 11.009
	(4)11.108	(4) 11.073
	Av. 11.006	Av. 11.030

SULPHURIC ETHER.

10 hour extraction of two five hour periods.

o/o of oil extracted in 1st 5 hrs.	o/o of oil extracted in 2nd 5 hrs.	Total o/o
11.568	.164	11.732

o/o of oil 10 hour extractions	o/o of oil 16 hour extractions.
(1) 11.667	(1) 11.666
(2) 11.460	(2) 11.484
(3) 11.448	(3) 11.471
(4) 11.257	(4) 11.606
(5) 11.545	
Av. 11.567	Av. 11.557

MODIFIED METHOD.

	o/o of oil 1st blow-off	o/o of oil 2nd blow-off	o/o of oil 3rd blow-off	o/o of oil 4th blow-off	Total o/o
(1)	10.146	.508	.149	.019	10.822
(2)	10.152	.459	.099	.029	10.739
(3)	9.991	.489	.089	.029	10.589
(4)	10.135	.538	.169	.015	10.857
(5)	9.982	.519	.150	.019	10.670
Av.	10.081	.503	.131	.022	10.737

Conclusions:

The above modified method for the determination of oil in cereals appears to give very satisfactory results compared with those obtained by means of petroleum ether as a solvent. There are several favorable features which make its employment desirable.
(1) It is a short method, three and one half to four and one half hours sufficing for complete extraction.
(2) It obviates difficulty of removal of last trace of solvent.
(3) It produces a purer oil, and an oil practically free from insoluble matter which is always extracted by ether.
(4) It is a good working commercial method.