The Determination of the True Iodine Numbers of Oils Containing Conjugated Double-Bond Systems[1]

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THE halogen absorption methods now in general use are satisfactory with oils containing only single ethylenic linkages and isolated double-bond systems. They are known to be unsatisfactory for determining the total unsaturation of fats and oils containing conjugated double bonds, such as tung oil and oiticica oil.

The work of Gelber and Boeseken (2) showed that the Wijs reagent adds quickly to one of the two conjugated double bonds in 9,11-linoleic acid, or two out of the three conjugated double bonds in eleostearic acid while the remaining double bond becomes saturated only after the reaction time has been extended from 2 to 6 days.

The Wijs method, when used with tung oil, gives a value above the partial saturation, where two of the three conjugated double bonds of eleostearic glyceride have reacted with the reagent. Van Loon (8) called this resulting value "the apparent iodine number." Gelber and Boeseken (2) and van Loon (9,10) showed that it is possible to determine the true total iodine number of conjugated oils using Wijs solution if the reaction time is extended to one week while using up to 700% excess reagent. Gelber and Boeseken obtained iodine numbers of 240-250 for tung oil while van Loon's values for tung oil ranged from 220-227. As a practical method of analysis such a procedure is much too slow.

The Rosenmund-Kuhnhenn method was shown by Kubelka and co-workers (7) and by McKinney and Jamieson (12) to give values for tung oil approximating the two-thirds or partial saturation point. McKinney and Jamieson found that with the Rosenmund-Kuhnhenn reagent this two-thirds reaction is independent of concentration and complete in 30 minutes and has the advantage over the Wijs reagent, whose reaction is influenced by time, temperature, and excess of reagent.

With the Hanus method the results obtained on tung oil vary even more than most of the other methods with time, temperature, and sample weight. The Hanus method was shown by Kreikenbaum (6) and other investigators to give iodine numbers for tung oil ranging from 189 to 211, and they therefore considered this method inapplicable to Chinese wood oil. Kubelka and co-workers (7) showed that the effect of excess reagent and reaction time on the Hanus iodine number of tung oil varied even more than the Wijs method. They concluded that using the Hanus reagent, the "true" iodine number of tung oil, employing a three-hour reaction time, was 243.4. McKinney and Jamieson (12), using the Hanus reagent, found the "true" iodine number of a Florida tung oil to be 246.9.

Von Mikusch and Frazier (14) have recommended the use of Hanus solution, in which the concentration of iodine bromide is about doubled, for determining the total unsaturation of oils and fatty acids containing conjugated double bonds. They found that with tung oil an iodine value of about 225 is obtained if temperature, excess reagent, and time of reaction are kept within certain narrow limits. The effect of changes in these factors gives considerable differences in the value for the total unsaturation of tung oil, much like the effect of similar changes in the determination of the Wijs iodine number of this oil.

Bromine vapor has been used to measure the absorption of all double bonds, including those conjugated. Various investigators using the bromine vapor method have obtained values for tung oil ranging from 217 to 266 (14). This method is very complicated and requires special equipment.

Need for a Simple Method. It is apparent that there is need for a simple method of determining the total unsaturation of conjugated fats and oils. The apparent iodine number of tung oil as determined by the standard methods is so little representative of its quality and performance that practical tests such as gelation tests are usually employed in its evaluation.

The use of the total iodine number of tung oil, having the highest total unsaturation of all the oils, would move tung oil to the top of the list where it belongs. In addition, conjugated double bonds which are known to be the most effective for fast drying would no longer appear at a discount in the iodine number of the oil. Adulterant oils having iodine numbers near the apparent iodine number of tung oil would have a considerable effect on the total iodine number and become easier to detect. Changes due to oxidation or polymerization would then become measurable as a decrease in the total iodine number whereas now the partial and apparent iodine numbers are little affected and in a few cases increase. Finally, the new synthetic oils and fatty acids which are being developed to contain conjugated double bonds require a method for determining total unsaturation to effect proper control in the making of such products.

Studies on Tung Oil. The use of pyridine sulfate dibromide in conjunction with mercuric acetate has been shown by Benham and Klee (1) to increase greatly the rate of halogen absorption by non-conjugated fats and oils. Preliminary tests on tung oil, using the modified method, yielded the highest iodine number of any of the other halogen solutions used. Experiments were then performed to determine whether longer reaction time and greater excess reagent would result in complete saturation of all the double bonds present in tung oil.

The general procedure was used for all determinations. During preliminary tests it was noted that the presence of light during absorption affected the results to a slight degree. Therefore, as a precautionary measure, the absorptions were allowed to take place in the dark.

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TABLE I

Relation of Size of Sample, Excess Reagent, and Reaction Time to the Iodine Number of Tung Oil

Sample wt., grams	1 Minute		30 Minutes		60 Minutes		2 Hours		48 Hours	
	Iodine No.	Excess reagent, %	Iodine No.	Excess reagent, %	Iodine No.	Excess reagent, %	Iodine No.	Excess reagent, %	Iodine No.	Excess reagent, %
0.0443	230.5	530	250.4	472	250.4	472	251.2	475	250.9	477
0.0606	229.5	356	249.5	318	250.7	316	250.7	316	250.7	316
0.0808	225.2	244	246.1	219	249.3	214	252.3	217	251.7	216
0.1019	215.8	190	238.2	162	248.3	152	251.8	152	252.1	150
0.1220	207.0	145	237.1	115	247.4	111	248.0	110	248.6	109
0.1589	198.2	101	234.0	71	240.1	67	249.4	64	250.2	62
0.2030	187.0	68	219.9	43	224.4	40	238.5	34	249.8	28

The following samples of tung oil were used:

1. Sample of fresh tung oil, courtesy of B. Falk and Company.
2. Sample of tung oil, several years old, stored without precaution against oxidation.
3. Sample of fresh Chinese tung oil, courtesy of The Glidden Company.
4. Sample of fresh tung oil, courtesy of Sherwin-Williams Company.
5. Sample of fresh Florida tung oil, courtesy of U. S. Department of Agriculture, Gainesville, Fla.

Experiments were conducted on Sample 1 to study the relation of size of sample, excess reagent, and absorption time on the iodine number of tung oil. The results as tabulated in Table I show that when approximately 65% excess reagent and a 2-hour reaction time are used, the modified method gives reproducible figures for the iodine number of tung oil, Sample 1. This sample which had an apparent iodine number of 156.7 by the Rosenmund-Kuhnhenn method, gave constant values, within the experimental error averaging 250.6, with the modified method using a 2-hour reaction time and 65 to 475% excess reagent. Where speed is most important, the use of sample weights below 0.06 grams, using 325-475% excess reagent, results in constant iodine numbers when a reaction time of 30 minutes is allowed.

The iodine numbers of the various tung oils were determined by the modified method, using a reaction time of 2 hours and 65 to 100% excess reagent. From the results listed in Table II, it is evident that changes in tung oil due to oxidation and/or polymerization on long standing may be more easily detected by the modified method than by the Rosenmund-Kuhnhenn method. Sample No. 2, several years old and stored without precaution against deterioration, had an iodine number of 242.5, a difference of approximately 8 units from the iodine number of a fresh sample of tung oil. On the other hand, the Rosenmund-Kuhnhenn method yields iodine numbers for fresh and old tung oil which differ by only 1 or 2 units. Similar results are obtained by the other general methods now in use.

TABLE II

Iodine Numbers of Tung Oil

(Reaction time, 2 hrs., excess reagent, 65-100%)

Sample	Iodine Number	
	Modified method	Rosenmund-Kuhnhenn method
1	250.6	156.7
2	242.5	154.3
3	250.2	155.9
4	251.3	157.8
5	247.5	155.1

Absence of Secondary Reactions. The following experiment was performed to determine whether substitution reactions might be the cause for the high iodine number (250.6) obtained for tung oil when pyridine sulfate dibromide in conjunction with mercuric acetate was used. Two identical quantities of tung oil (0.1090 gm.) were each subjected to the action of 50 ml. of the pyridine sulfate dibromide reagent plus 10 ml. of the catalyst for 48 hours. To one sample potassium iodide and water were added, and the iodine number as determined in the usual manner was found to be 250.2. To the other sample was added 100 ml. of water, and the mixture was extracted repeatedly with 30-ml. portions of chloroform until all the free bromine was removed, leaving the halogen acid in the aqueous layer. This procedure was repeated with blank determinations, using 50 ml. of reagent and 10 ml. of catalyst. In all cases the separated aqueous solutions were acidified with nitric acid, the halogens precipitated with 10% silver nitrate solution and then filtered, dried, and weighed.

It was found that the aqueous solution of the blank determination yielded 0.1216 gm. of silver bromide while that of the tung oil determination yielded 0.1204 gm., a difference of only 0.8 mg. Duplicate determinations gave similar results. These results are evidence of the absence of any secondary substitution reactions and substantiate the findings of Rosenmund and Kuhnhenn (15) and Rowe, Furnas, and Bliss (16) in this respect.

Studies on Conjugated Compounds

Oiticica Oil. It has not been possible hitherto to find a method to give any reproducible iodine numbers to this oil. Kaufmann and Baltes (5) ascribe the difficulties to the presence of the keto group which "interferes so much with the iodine test that the analyst finds it impossible to determine any iodine number at all." However van Loon (11) has reported that the Wijs iodine number of both oiticica and poyoak oil, with large excess of reagent, becomes constant after one week, as in the case of tung oil. As a practical method of analysis such a procedure is inadequate because of its slowness.

Two samples of oiticica oil were tested by the modified method using varying reaction time and 130 to 160% excess reagent. Sample 1 which had an apparent iodine number of 144.8 by the Wijs method with 320% excess of reagent for 30 minutes, gave constant values, within the experimental error averaging 216.5, by the modified method using 155% excess reagent and a 2-hour reaction time (Table III). This value is in agreement with the total iodine number of oiticica oil found by McKinney and Jamieson (13) to be 217.5. Sample 2, using the modified method, was found to have an iodine number of 205.5. with a corresponding lower Wijs iodine number of 135.0.

TABLE III

Iodine Numbers of Conjugated Compounds

Sample	Excess reagent,%	Time, hrs.	Iodine Number	
			Found	Calculated
Beta-eleostearic acid	350	1	272.1	273.7
	350	2	273.3	273.7
	350	48	272.8	273.7
90% Tung oil plus 10% soybean oil	350	1	237.9	238.8
	350	2	239.0	238.8
	350	48	239.0	238.8
80% Tung oil plus 20% soybean oil	350	1	228.1	227.4
	350	2	228.7	227.4
	350	48	228.7	227.4
Oiticica oil, Sample 1	160	1	215.8	....
	155	2	216.5	....
	150	48	217.3	....
Oiticica oil, Sample 2	130	1	205.0	....
	130	2	205.5	....
	130	48	205.9	....

Beta-Eleostearic Acid. This acid was prepared according to Thomas and Thompson's procedure (17) and had a melting point of 71.5°C. The values obtained and the conditions used are listed in Table III. The close agreement of the iodine numbers found experimentally with the calculated theoretical iodine number of eleostearic acid is evidence of the absence of secondary reactions and further corroborates the conclusions of Rosenmund and Kuhnhenn and Rowe, Furnas, and Bliss, referred to previously.

Adulterated Tung Oil. Tung oil sample no. 1 was adulterated with 10 and 20% of soybean oil to study the effect of the adulterant oil on the resulting iodine number, using the modified and Wijs methods. Soybean oil (Iodine No. 128.6) was used as the adulterant since it has been used commercially for that purpose and also because it has an iodine number near the apparent iodine number of tung oil.

It was found that using the modified method, a 10% adulterated tung oil had an iodine number of 239.0, as compared to 250.6 for pure tung oil while the Wijs method yielded values of 159.7 and 163.0 for 10% adulterated and pure tung oil, respectively. Similarly, a 20% adulterated tung oil yielded iodine numbers of 228.7 using the modified method, and 156.4 using the Wijs method. A 10% adulterated tung oil is represented by a decrease of 11.6 units in the modified method iodine number, with a decrease of only 3.3 units in the Wijs iodine number. Similarly, a 20% adulterated tung oil represents a decrease of 21.9 units in the modified method iodine number, with a decrease of 6.6 units in the Wijs iodine number. From these observations it is apparent that the modified method is far superior to the Wijs method for the detection of adulteration of tung oil. The total iodine number of adulterated tung oil by the modified method changes considerably while the partial iodine number by the Wijs method is very little affected by the addition of the adulterant oil.

Dehydrated Castor Oil. In the dehydration of castor oil one hydroxyl group and a neighboring hydrogen are removed from the carbon chain of the ricinoleic acid to form a new double bond. Ricinoleic acid contains one double bond and dehydration can result in the formation of two new products. One can contain two double bonds in isolated positions, and the other can contain two conjugated double bonds.

Dehydrated castor oils vary in the amount of conjugation produced by the dehydrating process. It is necessary to use a method with dehydrated castor oil which will react with all the double bonds present, isolated as well as conjugated. The Rosenmund-Kuhnhenn, Wijs, and Kaufmann methods give iodine numbers which do not indicate the total unsaturation. Hanus solution when used in excess of at least 400% for 1 hour at 20°C. will give reproducible results which correspond fairly closely to the total unsaturation as calculated from the drop in hydroxyl value during dehydration.

A sample of dehydrated castor oil (Castung 403-GH) was tested by the modified method using varying sample weights. The theoretical total unsaturation of this oil is approximately 150 to 160, depending on the extent of dehydration. This sample which had an apparent iodine number of 138 by the Wijs method for one hour reaction time, gave constant values, within the experimental error averaging 159.5, with the modified method, using 225 to 700% excess reagent and a reaction time of one hour.

Another sample of dehydrated castor oil (Castung 403 Z-3) was tested by the modified method. This sample which had an apparent iodine number of 122.5 by the Wijs method, gave values averaging 145.7 by the modified method, using a 1-hour reaction time and 250 to 300% excess reagent.

Isomerized Soybean Fatty Acids. Fatty acids with conjugated double bonds made by an isomerization process from natural fatty acids give low iodine numbers with the standard methods. Since the ordinary iodine reagents measure all isolated, but only a portion of the conjugated double bonds, the apparent iodine number drops during isomerization.

A commercial sample of conjugated soybean fatty acids (Conjusoy), was evaluated by the modified method, using varying sample weights. This sample which had an apparent iodine number of 117.5 by the Wijs method with 390% excess reagent for one hour, gave constant values, within the experimental error, averaging 138.5 with the modified method using 150 to 640% excess reagent and a reaction time of one hour (Table V). This value is identical with the iodine number of the fatty acids before isomerization.

TABLE IV

Total Unsaturation of Dehydrated Castor Oil
(Theoretical total iodine number 150-160,
Wijs iodine number 138)

Weight of sample, grams	Excess reagent,% (Time, 1 hour)	Iodine number
0.0500	702	159.5
0.0608	578	160.4
0.0754	434	159.2
0.0920	336	159.2
0.1001	301	159.5
0.1202	228	159.4
0.1509	171	156.8
0.1840	122	156.5
0.2684	50	156.4
	(Time, minutes) (excess reagent 250-300%)	
0.1202	1	155.8
0.1158	15	156.7
0.1079	30	157.2
0.1196	60	159.4
0.1202	120	160.0

Summary

The modified Rosenmund-Kuhnhenn procedure, as described by Benham and Klee, has been applied to conjugated systems. The only difference is that the 1-minute reaction time is extended to 30-120 minutes

TABLE V
Total Unsaturation of Conjugated Soybean Fatty Acids
[Theoretical total iodine number 138.0, Wijs iodine number (390% excess) 117.5]

Weight of sample, grams	Excess reagent, % (Time, 1 hour)	Iodine number
0.0598	640	137.8
0.0893	386	138.6
0.1183	268	139.0
0.1196	265	138.5
0.1786	148	137.2
0.2366	94	131.8

depending upon sample weight and excess reagent. Such a procedure gives true iodine numbers and has been investigated for various tung oils, oiticica oil, isomerized fatty acids, and dehydrated castor oil.

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Extraction and Evaluation of Oil From Dried Brewers' Grains[1]

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THE principal by-product of the brewing operation is brewers' grains, which consist of the insoluble material remaining after mashing of the malt and adjuncts. The average analysis for these grains is as follows: moisture, 10%; protein (N × 6.25), 27.0%; crude fiber, 15.5%; ash, 3.5%; crude lipids, 7.0%.

Although the oil content of dried brewers' grains is relatively low, it was thought that these grains might serve as a commercial source of vegetable oils. In order to determine the chemical characteristics of this oil and the feasibility of extracting and refining it, the work reported herein was undertaken.

The first problem considered was the effect on the feeding value in dairy cattle rations if the grains were relatively fat-free. Evidence in the literature is somewhat contradictory on this point. For example, Monroe, *et al.* (1), concluded, "under the conditions of these trials no significant differences were observed in the production of milk, butterfat, or 4% milk, or in the general health of milking cows from the feeding of practical grain mixtures ranging in average fat percentage from 4.89 to 2.69." Maynard, *et al.* (2), reported a somewhat different view, namely, "in a continuous experiment involving two groups of eight cows each, the group fed the high-fat mixture (7% fat) produced 4.4% more milk, 2.0% more fat, and 4.1% more fat-corrected milk than did the group fed the concentrate mixture containing only 3% fat." Inasmuch as the proposed extraction of oil from dried brewers' grains could be expected to lower the fat content of the mixed feed ration by an average of only 0.9%, it was felt that the decrease would not significantly affect the feeding value of the mixed feed ration. Furthermore modern tendency is towards extraction in the oilseed industry, and a variety of extracted oilseed meals are now being accepted by feed manufacturers.

An analysis of the oil from spent barley malt has been reported by Täufel and Rusch in 1929 (3), but this work was performed on a European spent barley malt which contained no adjuncts. The present work was performed on spent grains derived from a mixture of American barley malt and rice. It is obvious that the analyses of the oil extracted from such residues will vary somewhat from brewery to brewery because of the use of different adjuncts, such as corn grits, corn flakes, corn syrup, milo maize, wheat flakes, and others. Since most breweries use a high ratio of barley malt to adjunct, the differences in the analyses of the oil from spent grains should not be pronounced.

Our work on this oil was roughly divided into three catefories, namely: a) methods of extraction, b) chemical analysis, and c) refining, which will be discussed in this order.

Methods of Extraction

It has been found in our laboratories that the average crude fat (that is, diethyl ether extractable by A.O.A.C. procedure) (4) of spent barley malt is 7.0% (as is basis), with a minimum of 5% and a maximum of 9%. The only practical way to recover this fat is by solvent extraction. For this purpose commercial hexane (boiling range 143° to 158°F.) was employed because of its wide use in the vegetable oil extraction industry.

Pilot studies were run on two continuous countercurrent extractors, namely, a horizontal and vertical type. The former type has been described by Lerman, *et al.* (5). The grains enter at one end of the extractor and are conveyed from section to section by impeller blades; the solvent enters at the other end and is pumped to the end that the grains entered.

The vertical extractor embodies the same principle of countercurrent extraction, that is, the fresh solvent is in contact with extracted marc, while the

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