

Determination of True Iodine Number of Oils with Conjugate Unsaturation by Use of Hypochlorous Acid Reagent

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NUMEROUS halogen absorption methods are available for measuring the unsaturation of oils and fats, and while a majority of them are satisfactory with systems containing isolated double bonds, they fail to record true values of unsaturation in the case of conjugated double bond systems. The incorrect figures arise from the incomplete absorption of the halogen reagents employed. It has been shown by Gelber *et al.* (4) that Wijs' reagent adds quickly to one of the two double bonds in 9,11-linoleic acid and to two of the three conjugated double bonds in eleostearic acid. The halogen absorption is only complete after standing for two to six days. By studying the effect of time, temperature, and excess reagent van Loon was able to determine the true iodine number of tung oil by using a 700% excess reagent and extending the contact time to one week (19). Because of the extreme slowness of this reaction, the method could not be employed on a practical scale in commercial laboratories. The use of Rosenmund-Kuhnheim and Margosches' reagent (15) was found to give values for tung oil approximately two-thirds of the total unsaturation.

The methods of Hubl, Wijs, and Hanus were applied to tung oil by Kreikenbaum (8), who found that it gave uniform results between 169-171. Kubelka *et al.* (9) showed that the effect of excess reagent and reaction time on the Hanus iodine number of tung oil varied even more than Wijs' numbers. Bromine has also been employed for the determination of total unsaturation in tung oil by Toms (18), Bolton and Williams (2), Levy (10), Rossmann (16), Boeseken and Pobs (1), and Brocklesby and Harding (3). None of these methods is a measure of true unsaturation. Kaufmann (6) used ultraviolet light to catalyze the addition of bromine to the third double bond in eleostearic acid. Quantitative hydrogenation has however been successfully applied to β -eleostearic acid and tung oil by Jordon (5), Brocklesby and Harding (3), and more recently by Pack *et al.* (13). It is considered to yield the most accurate results as there is no possibility of substitution or the side reactions which frequently complicate the bromine addition procedures. Hydrogenation procedure has the disadvantage that special equipment is required and can hardly compare in simplicity with the ordinary volumetric iodine absorption procedure. The modified Wijs procedures of Gelber and Boeseken or of van Loon require an undue length of time. The need for an ultraviolet lamp complicates the Kaufmann procedure.

There is at present a steadily increasing demand for a simple method for the quantitative determination of the total unsaturation of oils containing conjugated double bonds, which will be adaptable for routine work, will require no special apparatus, and will be applicable to both conjugated and non-conjugated systems. The apparent value for unsaturation of tung oil as determined by standard recommended official procedure is of little value in its evaluation,

and the performance of special practical tests, such as gelation time, is depended on for the determination of its quality. The need for a method for the true iodine number determination will be apparent from the fact that such procedure will be useful in readily detecting the adulteration of tung oil with oils having iodine values near the apparent iodine value of tung oil. The freshness of the oil and the changes in composition due to oxidation and polymerization effect, which would little affect the apparent iodine number of tung oil, would be readily detectible. The most important advantage will be the estimation of the amount of conjugate unsaturation in oils when this procedure is used in combination with partial iodine value, without the use of a spectrophotometer. Finally the new synthetic drying oils and fatty acids which are being developed to contain conjugated fatty acids require a method for determining total unsaturation for effecting proper control in the making of such products.

The first successful attempt was that of von Mikusch and Frazier (11), who employed the Hanus solution of 0.4 N IBr concentration and a 3-hr. reaction time at 0°C. and, using a 600% excess reagent, obtained values of 225-230 for tung oil. Klee and Benham (7) applied a modified Rosenmund-Kuhnheim procedure for the determination of true iodine number of oils containing conjugated double bonds, and they were able to get consistent results for tung, oiticica oil, DCO, and isomerized fatty acids by extending their 1-min. reaction time to 30 to 120 min., depending on sample weights and excess reagents employed. The experimental value of 250.2 obtained by the Benham-Klee procedure appears to be rather higher than that which can be expected from its fatty acid composition. Plank, Pack, and Goldblatt (14) were able to show that if the mercuric acetate reagent is added prior to the addition of the Rosenmund-Kuhnheim reagent in contrast to the Benham-Klee modification, in which the Rosenmund-Kuhnheim reagent is added first, and at the same time if care is taken to exclude light, consistent and reproducible results are obtained for tung oil.

Experimental

The highly encouraging results obtained with hypochlorous acid reagent in the determination of unsaturation of oils and fats having isolated double bonds by one of us (12) prompted the present investigators to extend the use of hypochlorous acid reagent for the estimation of conjugated unsaturation in natural oils by prolonging the time for absorption of the reagent. Imported Chinese tung oil procured from local paint manufacturers, having an iodine number of 241.6 (catalytic hydrogenation), was employed. The following table records the results of experiments conducted with tung oil for studying the relationship of sample size, excess reagent, and absorption time on the iodine number of the oil. It is evident that, even with the use of a 2-hr. absorption period, the addition of hypochlorous acid to the double bond is not com-

TABLE I
 Effect of Variation of Sample Weight and Excess Reagent on Iodine Number of Tung Oil by HOCl Reagent

Strength of hypochlorous acid	Sample wt. g.	15 min.		30 min.		60 min.		2 hrs.	
		Iodine Number	Excess Reagent	I.N.	Excess Reagent	I.N.	Excess Reagent	I.N.	Excess Reagent
0.1 N	0.0862	216	443	221	440	223	436	229	433
	0.1325	193	238	201	233	214	271	216	260
	0.1020	210	200	214	193	217	137	222	179
	0.1875	185	115	190	109	194	102	197	96

plete though increasing values were obtained with increase in absorption period; with relatively large sample weights and lower percentage of excess reagent, decreasingly lower values for the iodine number are obtained.

Effect of Increasing the Concentration of Hypochlorous Acid Reagent. It will be clear from the results of Table II that an increase in concentration of the hypochlorous acid reagent results in gradual increment in the iodine number, but even in presence of a considerable excess of reagent (400%) desirable results are not obtained.

TABLE II

Effect of Increasing the Concentration of Hypochlorous Acid Reagent

Concentration of HOCl	Iodine Number			
	30 min.	Excess Reagent	60 min.	Excess Reagent
0.1 N	220	450	223	442
0.15 N	221	410	225	400
0.2 N	223	470	226	461
0.25 N	225	450	229	435
0.30 N	226	480	231.5	465

It is therefore imperative that, in order to effect complete saturation of the double bonds, the use of a suitable catalyst must be resorted to. It has already been shown by Klee *et al.* (7) that mercuric acetate is an excellent catalyst in the absorption of bromine by the ethylenic linkages. The same catalyst was tried with the hypochlorous acid reagent. The mercuric acetate catalyst was employed in the form of a solution in glacial acetic acid and, instead of using 10 ml. of glacial acetic acid, an equal volume of mercuric acetate solution was employed. The general procedure outlined previously was employed with no other modification.

Experiments were performed to study the relationship between sample size, time of absorption, and percentage of excess reagent with a fresh sample of tung oil, and it will be evident from the data in Table III that reproducible results are obtained, using a 60-min. reaction period and above 300% excess reagent. Even with less than 200% excess reagent, hypochlorous acid gives, within experimental error, constant values averaging 241-242 when longer absorption periods are employed.

For quick and reproducible results use of 0.07-0.10 g. of sample and 300 to 400% of excess reagent

are recommended with 1-hr. reaction period. With less time however, the absorption of hypochlorous acid reagent is incomplete even using 1,000% excess reagent.

It will be clear from the results shown in Table IV that this method can be advantageously employed in detecting changes in tung oil due to oxidation and polymerization on aging.

Tables V and VI record, respectively, the effects of variation of catalyst concentration and temperature

TABLE IV

	Iodine Number	
	HOCl Method	Wijs Method
Fresh tung oil	242.3	162.5
An old sample of tung oil	232.8	160.3

on the iodine number of tung oil, using hypochlorous acid reagent. It will be evident from Table V that increase in catalyst concentration from 0.5 to 2.5% is accompanied by a gradual increase in the iodine number of the oil. The use of larger amounts of the catalyst is limited by the solubility of mercuric acetate in glacial acetic acid. The results of Table VI indicate that, using a 60-min. reaction period and a 2.5% mercuric acetate catalyst, reliable values for the iodine number of tung oil are obtained in the neighborhood of 20°C. Slightly lower values are obtained at ordinary temperatures.

 TABLE V
 Effect of Variation of Catalyst Concentration on the Iodine Number of Tung Oil

Catalyst Conc.	% Excess Reagent	Iodine Number
0.5	280	237.9
1.0	360	238.2
1.5	365	240.0
2.0	310	242.5
2.5	400	242.8

 TABLE VI
 Effect of Variation of Temperature on Iodine Number of Tung Oil (using 2.5% mercuric acetate catalyst)

Time	Temperature	% Excess Reagent	Iodine Number
60 min.	0-5°C.	300	241.4
		275	241.7
60 min.	20° ± 2°C.	400	240.8
		400	241.7
60 min.	35-40°C.	400	238.2
		300	237.8

 TABLE III
 Estimation of Total Unsaturation of Tung Oil with HOCl Reagent in Presence of Mercuric Acetate Catalyst (2.5%, in glacial acetic acid solution) [10 ml. of mercuric acetate soln. + 25 ml. of 0.3 N hypochlorous acid solution]

Sample wt. g.	5 min.		10 min.		30 min.		60 min.		2 hrs.		48 hrs.	
	Iodine Number	Excess Reagent	Iodine Number	Excess Reagent	Iodine Number	Excess Reagent	Iodine Number	Excess Reagent	Iodine Number	Excess Reagent	Iodine Number	Excess Reagent
0.0445	198.4	1100	216.8	1060	237.0	1030	242.0	1020	242.6	1000	242.6	1000
0.0780	201.3	640	217.2	650	238.5	550	242.6	500	242.8	500	242.8	500
0.1085	201.8	380	215.2	360	237.0	350	242.2	310	242.6	300	242.6	300
0.1520	197.8	250	216.0	246	234.0	230	241.8	216	242.0	200	242.1	200
0.2020	200.8	227	215.6	214	234.6	200	241.8	190	241.8	190	242.2	180

The method was extended for the determination of iodine numbers of β -eleostearic acid and isomerized 9, 10-11, 12-linoleic acid. Beta-eleostearic acid was prepared according to the method of Thomas *et al.* (17), and linoleic acid was isomerized with 7.5% KOH in glycerine by heating in a constant temperature isomerization bath at 180°C. for 1 hr. The isomerized acid was extracted with ether from the acidified reaction product, the ethereal layer was washed and dried, the solvent was evaporated off in a current of nitrogen, and the iodine number was determined with 0.3 N hypochlorous acid reagent, using 1-hr. absorption time at 20°C.

TABLE VII
Iodine Number of Fatty Acids with Conjugated Double Bonds

	% Excess Reagent	Time	Iodine Number		
			Theoretical	Found	
				HOCl Method	Hydrogenation Method (13)
Beta-eleostearic acid.....	350 350 400 350	1 hr. 1 hr. 1 hr. 2 hrs.	273.8	273.7 273.2 273.5 273.5	272.3
9:10, 11:12, Linoleic acid....	395 400 400 400	1 hr. 1 hr. 1 hr. 2 hrs.	181.03	181.3 182.0 181.0 181.7	181.6

Detection of Adulteration of Tung Oil. Fresh tung oil was admixed with 10, 15, and 20% of Calcutta linseed oil (iodine value —172) to study the effect of the adulterant oil on the resulting iodine number, using the present method and the conventional Wijs procedure. Calcutta linseed oil was chosen because of its frequent use as an adulterant for the tung oil and also because its iodine number is near the apparent iodine number of tung oil.

It was found from the results in Table VIII that, using the hypochlorous acid reagent, a 10% adulterated tung oil had an iodine number of 236 as compared to 242.6 for the pure tung oil while the Wijs procedure gave values of 173.1 and 174, respectively, for the adulterated and pure tung oil. The difference was too small to be of any significance in evaluating the extent of adulteration. Even with samples adulterated with 15 and 20% of Calcutta linseed oil, the Wijs method does not show any marked difference whereas with the hypochlorous acid procedure the fall in iodine number is 6, 10, and 13 units when the pure oil is mixed with 10, 15, and 20% of the adulterant. The total iodine number thus changes considerably while the partial iodine value as determined by the Wijs method is very little affected by such adulteration.

TABLE VIII
Iodine Number of Adulterated Tung Oil

	Excess Reagent	Time	Total (Theo.)	Iodine Number		
				HOCl (Exptl.)	Wijs	
					Calc.	Exptl.
90% Tung Oil + 10% Calcutta Linseed.....	350 400	1 hr. 1 hr.	235.0 235.0	235.8 236.0	173.8	173.1
85% Tung Oil + 15% Calcutta Linseed.....	350 400	1 hr. 1 hr.	231.8 231.8	231.2 231.7	173.7	173.0
80% Tung Oil + 20% Calcutta Linseed.....	350 400	1 hr. 1 hr.	228.0 228.0	228.5 229.0	173.6	173.2

Dehydrated Castor Oil. Samples of unboiled dehydrated castor oil were tested by the new method, using varying sample weights. The theoretical total unsaturation of this sample was calculated to be 166 from the spectrophotometric analysis of its unsaturated fatty acid composition and unsaturation by the Woburn procedure (11). The sample, which had an apparent iodine value of 141.2 by the Wijs procedure, gave constant values within experimental error, averaging 165-166 with a 300% excess of 0.3 N hypochlorous acid reagent by using 1 hr. reaction time at 20°C.

TABLE IX
Total Unsaturation of Dehydrated Castor Oil
(theoretical total iodine number 165-166; Wijs iodine number—141.2)

Sample Weight g.	Excess Reagent % (Time—1 hr.)	Iodine Number	
		Excess Reagent	Time
0.0512.....	740	1 hr.	165.8
0.0750.....	450		166.0
0.1098.....	300		166.2
0.1212.....	238		165.6
0.1738.....	140		165.0
0.2010.....	82		164.3
0.1600.....	340	1 hr.	166.0
0.1658.....	325	30 min.	164.9
0.1652.....	330	15 min.	164.7
0.1650.....	350	5 min.	161.3

This table also shows iodine values obtained with HOCl reagent when the time of contact was varied from 5 to 60 min. These data indicate that over 80-85% of the addition takes place within first 5 to 10 min., and constant values are obtained only after a 60-min. contact time.

Comparison of Iodine Number of Tung Oil by Different Methods. In Table X is shown the iodine number of tung oil obtained by the various methods.

The result by the hypochlorous acid method agrees fairly well with the catalytic hydrogenation procedure and is comparable with the other accepted methods for the determination of total unsaturation of tung oil.

Summary

Hypochlorous acid reagent has conveniently been used for the determination of total unsaturation in oils containing conjugated double bonds. The use of 0.1 N HOCl reagent normally employed for oils with

TABLE X

Method	Iodine Number
Wijs.....	162.0
Woburn.....	239.8
Benham-Klee.....	249.3
Plank-Pack-Goldblatt.....	241.8
Hypochlorous Acid.....	242.1
Catalytic Hydrogenation (13).....	241.6

isolated double bonds leads to incomplete absorption, and the increase in concentration and reaction period gives desirable results in the presence of mercuric acetate catalyst. A 1-hr. reaction period with 0.3 N HOCl reagent in the presence of 2.5% solution of the catalyst is recommended. A sample size varying between 0.07-0.1 g. and a 300-400% excess reagent should be employed to obtain reliable results. This procedure can be effectively used for determining the total unsaturation of tung oil, isomerized fatty acids, and dehydrated castor oil and can be employed for detecting the adulteration in commercial samples of tung oil, which cannot be ordinarily detected by the determination of the partial iodine number with the help of conventional procedures.

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Carbonyl Compounds in Rancid Corn Oil

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IT WAS NOTED by Brekke and Mackinney (1) that steam distillates of rancid corn and avocado oils, collected in 2,4-dinitrophenylhydrazine solution, yielded a complex mixture of hydrazones (DNPHs). These were separated on the basis of solubility in petroleum ether, in which the common dicarbonyl and short chain alkyl derivatives are virtually insoluble, followed by chromatography on anhydrous magnesium sulfate. Fractions with absorption spectra corresponding to saturated aldehydes and to 2-enals and 2,4-dienals [cf. Braude and Jones (2)] were thus isolated. It was concluded that the corn oil DNPH mixture was comprised of at least five components soluble in petroleum ether, together with an uninvestigated petroleum ether-insoluble (benzene-soluble) fraction.

A more intensive study has now been made of the DNPHs obtained from rancid corn oil by the procedures already described, and 14 components have been separated in the petroleum ether-soluble fraction, as well as 16 from the P.E.-insoluble fraction. Two components have been isolated in quantity sufficient for elementary analyses and mixed melting point determinations with known compounds.

The data illustrate the advantages and definite disadvantages of techniques for separating dinitrophenylhydrazine derivatives in a really complex mixture.

Experimental

A commercial refined corn oil was subjected to accelerated rancidification by heating and aeration. It was then steam-distilled, and the carbonyls in the distillate were converted to 2,4-dinitrophenylhydrazones, which were separated by chromatography and their properties studied.

Methods

Rancidification. The corn oil, in three lots of a pint each, was held in a one-liter round bottom flask for

10 days at temperatures between 78° and 85° with air drawn through by suction from an aspirator at a rate of 40 to 60 bubbles per minute. The progress of rancidification was followed by determinations of the peroxide value of the oil.

Steam Distillation. Steam was generated in a two-liter flask short-coupled to the oil flask, admitting the steam through a glass tube extending to the bottom. To reduce condensation, the oil flask was kept in a boiling water bath during distillation. A water-cooled condenser received the distillate; its tip led below the surface of reagent in a flask.

Conversion of Carbonyl Breakdown Products to DNPHs. A saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl was employed in the receiving flask, precipitating red-orange derivatives. Successive 25-ml. portions of the reagent in 125-ml. Erlenmeyer flasks were used, substituting a fresh flask while there was still an excess of unreacted reagent in the supernatant of the previous flask. Initially about 25 ml. of distillate were collected with each portion, but after the greater part of the carbonyl compounds had been distilled, quantities up to 60 ml. were collected each time. From each pint lot of rancidified oil a total of 2,500 to 3,000 ml. of distillate was obtained. Precipitate was still forming in the receiving flask in small amount when distillation was stopped.

The precipitated DNPHs were washed repeatedly by decantation, first with hot 2 N HCl to remove unreacted DNP hydrazine, then with distilled water to neutrality. After drying under vacuum at temperatures not exceeding 40°C., the material was dissolved in benzene, yielding a deep red solution.

Preliminary Separation. To remove the DNPHs soluble in light petroleum ether (P.E.), 10 to 15 volumes of P.E. were added to successive portions of the benzene solution, and the precipitated P.E.-insolubles were filtered with Whatman No. 1 paper previously washed with benzene and P.E. The filter paper con-