A Rapid Method for the Estimation of Unsaturation of Fats and Oils by Use of Hypochlorous Acid Reagent

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THE DETERMINATION of iodine number for the measurement of the degree of unsaturation in fats and oils has been of such universal importance that numerous halogen absorption methods have been proposed for this purpose and although a majority of them are satisfactory with oils containing only single ethylenic linkages, only those of Wijs (12) and Hanus (5) have found extended application. The former procedure is recommended by the American Oil Chemists' Society and the latter by the A.O.A.C. Two other methods have often been employed by German workers, *viz.*, Kaufmann (8) and Rosenmund-Kuhnhenn (11), the latter with some advantage in biological laboratories. A comparison of the important methods reveals that while higher values for iodine numbers are obtained with the Wijs' method (for oil of iodine number above 100), the Rosenmund-Kuhnhenn and Kaufmann procedures give lower values (4).

Another distinctly striking feature is that all the methods specify long absorption time, 30-60 min. and some times 2 hrs. (Kaufmann), this time being too long for rapid control of continuous plant operations and sometimes very inconvenient. The use of catalysts such as $HgCl_2$ to reduce the absorption time was suggested by Hubl as early as 1884, and the same catalysts were used by Hoffmann and Green (6) for shortening the time of reaction, using the Wijs procedure. Other workers (Norris and Bushwells) reduced the absorption time to 3 min. with the Hubl method (7). Benham and Klee (2) have further modified the Rosenmund-Kuhnhenn method by use of mercuric acetate catalyst, and the reaction time has been reduced to only 1 min. It is the purpose of this paper to present a rapid method for estimation of unsaturation of oils by using aqueous solution of hypochlorous acid as the absorption reagent.

The addition of hypochlorous acid to ethylenic linkage with the formation of chlorohydrin was investigated by Albitzky (1), Nicolet and Poulter (10), and it was shown that β-methyl isobutylene gives a quantitative yield of the corresponding chlorohydrin when excess of reagent is used. This reaction was utilized by Goswami and Basu (3) in determining the unsaturation of sodium salts of fatty acids with hypochlorous acid. The method has been successfully employed by Mukherjee (9) for estimation of semi-micro quantities of fatty acids in aqueous solutions of sodium salts of fatty acids. So far the workers have employed aqueous solution of sodium salts of fatty acids for the estimation, but the present paper attempts the absorption of hypochlorous acid reagent with the glycerides themselves.

In our work the use of customary oil solvents like chloroform, carbon and tetrachloricle, employed in determination of iodine number, could not be made as the use of aqueous solution of NaOCl and glacial acetic acid to the $CHCl_3$ solution of oil led to incomplete absorption of the reagent. It was found that the reaction would be conveniently carried out by employing either an acetic acid solution of the oil or a suspension of the oil in acetic acid as the case may be. The procedure then simplifies into weighing out the sample in a glass-stoppered bottle, adding glacial acetic acid, shaking to take the oil in solution (or form a suspension), and then adding the aqueous solution of NaOCl of requisite strength. The use of 5-10 ml. acetic acid is found always to liberate the free hypochlorous acid completely from the sodium salt. The bottle is allowed to stand for a determined interval and potassium iodine solution is added, and the liberated iodine is titrated with thiosulphate as usual.

Preparation of the Reagent. The hypochlorous acid reagent can be conveniently prepared by passing a stream of washed chlorine through a 4 N solution of caustic soda, maintained at a temperature below 10°, until 5 ml. of the solution required 30-35 ml. of 0.1 N sodium thiosulfate solution for titrating its iodine equivalent. It takes 1 hr. to 1½ hrs. to prepare about 2 litres of the reagent. The hypochlorite reagent is stored in amber-colored bottles in a refrigerator. The solution is diluted to 0.1 N immediately before use. It has been observed that the more concentrated solutions keep better than the dilute solution. The reagent is very stable and keeps for several months.

The acetic acid used must be free of oxidizable impurities; the A.R. grade acid serves the purpose very well.

Experimental

In the first place, a study of the effect of time and use of excess reagent on the absorption of HOCl by the oil was carried out. As it appears from previous studies with hypochlorous acid reagent (9) that sample size is relatively unimportant so long as large excess of reagent is used, the weights employed in these studies was of the same order as recommended for the Wijs procedure.

Effect of Excess Reagent on the Iodine Number. Experiments were conducted with coconut, butter fat, peanut, castor, and sesame oils, using a fixed sample weight (0.1 to 0.12 g.), 10 ml. of acetic acid, and different volumes of the sodium hypochlorite reagent. The absorption time in each case was 30 min. The results are given in Table I.

It is apparent that when smaller amounts of excess reagent are employed, the absorption is not complete

TABLE I

Effect of Use of Excess HOCl Reagent on the Iodine Number of Oils

Excess reagent	Iodine Number				
	Coconut	Butter fat	Peanut	Castor	Sesame
%					
25	8.0	31.0	89.5	83.5	108.3
50	8.0	31.2	90.7	83.9	108.9
75	8.9	33.4	91.8	84.7	109.7
100	9.0	33.4	92.6	85.5	110.0
150	9.0	33.5	92.6	85.5	110.1
200	9.0	33.4	92.6	85.5	110.0
300	8.9	33.0	92.5	85.5	110.2
400	8.8	33.0	92.5	85.3	109.6
500	8.6	32.7	92.1	85.3	109.6

and the iodine number increases with increasing percentage of excess reagent and attains a more or less constant value near 100%. In case of the more saturated fats this constant is obtained even earlier, 75% excess being sufficient in case of coconut and butter fat. The lower values obtained in the last two cases were due probably to the undue dilution of glacial acetic acid employed by the larger volume of water accompanying such a large excess of reagent. This dilution effect can however be counterbalanced by use of appropriate quantities of glacial acetic acid (cf. Table IV) to maintain approximately the same acid concentration in each case.

Effect of Absorption Time on Iodine Number. Next, the effect of varying the reaction period, using a definite amount of excess reagent, was studied. The excess reagent used in these experiments was approximately 100-125% of the theoretical.

It is evident from Table II that the addition of HOCl to the double bond is extremely rapid, almost 85-90% of the addition taking place in course of first 30 seconds, and the absorption is complete in course of 3 to 4 min., irrespective of the fact that the oils examined were chosen from each of non-drying, semi-drying, and drying oil groups. The effect of prolonged reaction period has very little effect on the substitution of the reagent except in the case of castor oil, where although no marked effect is observed when using absorption period of 1 hr., the iodine number is rather high when longer absorption periods are employed (*e.g.,* 6 hrs.). The HOCl reagent thus possesses a great advantage since no secondary substitution reaction takes place within reasonably longer periods. In the case of castor oil containing hydroxy acids the rate of secondary reaction is too insignificant compared to the absorption of HOCl to the double bond, and very exact values are obtained when short reaction periods are employed. Hence for routine analytical procedure the time limit of 4 to 5 min. can be safely employed for accurate determination of iodine numbers of fats and oils in general.

Effect of Temperature. Owing to the Comparatively high rate of reaction between HOCl reagent and ethylenic linkages, the temperature coefficient would not be of much significance for the practical working out of the method. A study of the reaction was made at the following temperatures: 0-5°, 10°, 20°, 30°, 37°, and 40°C. (Table III). The results indicate slightly lower value near about the freezing temperature, but indicate identical values in the range 20-40°C. A reaction period of 5 min. was employed in each experiment.

TABLE III

Effect of Temperature on the Addition of HOCl to Oils

Temperature	Iodine number	
	Peanut oil	Butter fat
0 –5°C.	90.9	31.2
10°±1°C.	92.6	32.7
20°±1°C.	92.6	33.4
30°±1°C.	92.6	33.5
37°±1°C.	92.6	33.5
40°±1°C.	92.6	33.5

Effect of Acetic Acid Concentration on the Iodine Number. The following table records the effect of using different strengths of acetic acid in the determination of unsaturation by the HOCl absorption method; 5 ml. of 0.1 N NaOCl reagent were employed in each of the experiments.

The results of Table IV require careful consideration. In this process of determination of unsaturation no separate oil solvent is employed. The acetic acid that is used helps to keep the oil in solution. When the hypochlorite reagent is added, the oils are not thrown out of solution but provide a fine dispersion, giving a white turbidity to the reaction mixture. It has been found that instead of adding the acetic acid to the oil, if the hypochlorite reagent is added first, followed by acidification, no such fine dispersion is obtained and slight error in the experimental results occurs. The method takes advantage of the solubility of the oils in glacial acetic acid, but this latter factor depends also on the concentration of the acetic acid employed. This effect is clearly borne out by the data in Table IV, which show that lower values for iodine numbers are obtained when dilute acetic acid solution is employed for dispersion of the oil and acidification of the NaOCl reagent. It has been observed previously that slightly lower iodine numbers are obtained when the acetic acid solvent is unduly diluted by use of a large excess of NaOCl reagent (Table I). It is therefore imperative to study the effect of concentration of HOCl on the completeness of absorption of this reagent by the ethlynic linkages. It has been found that unless the strength of the HOCl reagent is lowered much below 0.02 N, the results are not moderately affected. A 0.1 N solution of NaOCl has been used throughout this investigation with very consistent and satisfactory results.

TABLE IV

Effect of Acetic Acid Concentration on the Iodine Number of Oils

Acetic acid, %	Vol. of acid	Peanut oil	Butter fat
	ml.		
50	10	70.3	25.8
60	10	74.5	27.6
70	10	81.2	30.4
80	10	89.5	32.6
90	10	92.0	33.5
100	10	92.6	33.5
100	5	92.3	33.5
100	7.5	92.6	33.5
100	20	92.6	33.5

TABLE II

Effect of Shorter and Longer Reaction Periods on the Absorption of HOCl by Fats and Oils

Oil	Iodine Number											
	Reaction Time											
	30 sec.	1 min.	2 min.	3 min.	4 min.	5 min.	7½ min.	10 min.	30 min.	60 min.	2 hrs.	6 hrs.
Coconut	6.1	6.8	8.2	8.9	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.1
Butter fat	30.9	31.8	32.8	33.4	33.5	33.5	33.5	33.5	33.5	33.5	33.6	34.0
Mustard (black)	99.0	100.1	101.0	102.0	102.3	102.3	102.3	102.3	102.3	102.3	102.3	103.0
Peanut	85.6	88.2	89.4	91.6	92.6	92.6	92.6	92.6	92.6	92.6	....	93.3
Cottonseed	108.0	109.2	110.6	111.8	112.0	112.0	112.0	112.0	112.0	112.0	112.0	112.8
Linseed	175.8	177.0	178.0	178.6	178.8	178.8	178.8	178.8	178.8	178.8	179.0	180.2
Castor	82.4	83.5	84.2	85.4	85.5	85.5	85.5	85.5	85.5	85.5	87.0	89.8

TABLE V

Comparison of Iodine Numbers Determined by Different Methods

Oil	Iodine Number Determined by				
	Wijs	Hanus	Rosenmund-Kuhnhenn	Benham-Klee modification of Rosenmund-Kuhnhenn	HOCl
Coconut	9.3	9.3	9.0	9.4	9.0
Butterfat	33.7	33.7	33.4	33.5	33.5
Castor	85.9	84.5	84.3	84.3	85.5
Olive	85.0	84.7	84.3	84.8	85.0
Peanut	92.5	92.3	92.2	92.5	92.5
Mustard (black)	102.8	102.0	102.0	102.5	102.5
Cottonseed	113.3	113.8	108.0	111.2	112.0
Linseed	183.0	179.0	178.0	179.0	179.0
Oleic acid	89.6	88.8	89.4	89.4	89.5
Linoleic acid	180.6	179.8	174.0	178.8	179.4
Tung oil	162.0	203.0	160.0	220.0	198.0

Comparison of Iodine Number of Oils by Different Methods. The iodine numbers of different fats and oils obtained by use of HOCl reagent were next compared with those determined by the standard procedure of Wijs (A.O.C.S. procedure), Hanus (A.O.A.C. procedure), Rosenmund-Kuhnhenn, and the modified Rosenmund-Kuhnhenn procedure of Benham and Klee. The survey of the results of Table V indicates that the HOCl method compares most favorably with the Hanus and modified Rosenmund-Kuhnhenn procedure; Wijs procedure giving slightly higher values. In the exceptional case of tung oil containing the diene eleostearic acid, the iodine number by the HOCl procedure lies intermediate between the modified Rosenmund-Kuhnhenn and the Hanus (or Wijs) method; but nevertheless the value is nearly 40 units higher than the Wijs procedure, thereby indicating the possibility that the true total unsaturation of this oil could be determined by increasing the time factor.

Recommended Procedure. The following procedure is recommended for the rapid determination of unsaturation of all non-conjugated oils and fats. The oil or fat is weighed out in a 500-ml. glass-stoppered bottle and 10 ml. of glacial acetic acid are added. The bottle is thoroughly shaken to dissolve the oil or thoroughly to disperse the oil in the acid, and 25 ml. of 0.1 N sodium hypochlorite reagent are added, the mixture is shaken well and allowed to stand in the dark for 4 to 5 min. at room temperature. Twenty ml. of 15% potassium iodine solution is next added, and after 1 min. the liberated iodine is titrated in the usual manner with 0.1 N sodium thiosulfate, using starch as indicator. A blank experiment is run simultaneously with the acetic acid and sodium hypochlorite reagent only. The iodine number is calculated as follows:

$$\text{Iodine number} = \frac{100 \times (b - a) \times f}{W}$$

b = ml. of 0.1 N sodium thiosulfate used for blank

a = ml. of 0.1 N sodium thiosulfate used for the oil or sample

f = mg. of iodine equivalent to 1 ml. of 0.1 N sodium thiosulfate solution

w = sample weight in mg.

The following table records sample weights suggested for various oils and fats in order that proper amount of excess reagents may be present when 25 ml. of the hypochlorite reagent are based, although it may be pointed out that even in the presence of considerable excess of the reagent (*viz.*, even up to 300%) no error in the experimental results would be observed.

TABLE VI

Recommended Sample Weights for Different Oils

Oils Having Iodine Number	Sample wt. in mg.
Below 50	500–1000
50–100	150–300
100–150	120–150
Above 150	100–120

Summary

A rapid method for the determination of unsaturation of oils and fats containing non-conjugated ethylenic linkages has been developed, using hypochlorous acid as the reagent. The method has several advantages, *viz.*, that the estimation is conducted with an aqueous solution of sodium hypochlorite reagent, and moreover no oil solvent is required for the estimation. In all the methods currently employed, the reagents used must be perfectly anhydrous otherwise incomplete addition will take place, giving low results. Since a reaction time of 4 to 5 min. is recommended, the estimations are much more rapid than the customary Wijs or Hanus methods. No discrimination as to time factor need be made for oils possessing different degree of saturation. All oils, whether belonging to non-drying or drying groups, give accurate results within the specified time. There is no secondary reaction except in the case of castor oil, but even in that case the secondary reactions are not appreciable within the first hour of reaction. The present method compares favorably with the standard procedures currently employed for determination of unsaturation.

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