Iodine Number Determination of Milk Fat and Vegetable Fats by Refractometry

M.K. RAMA MURTHY and **G.S. BHAT**, Dairy Chemistry Department,
Southern Regional Station of National Dairy Research Institute, Hosur Road, Bangalore 5600 30, India

ABSTRACT

A refractometric method for the estimation of iodine number of milk fat has been suggested. About 0.2 ml of milk fat was iodinated with ca. 10 ml Wijs iodine reagent for 3 min using mercuric acetate as catalyst. The iodinated fat showed a higher refractive index than the original fat. The changes in refractive indices showed a very high correlation with the iodine values of the fats ($r = 0.9993$). The average of the ratios of change in refractive index to iodine number was 50.7×10^{-5}, from which the iodine number of milk fat can be calculated. The method can also be applied to vegetable fats. The ratios of change in refractive index to iodine number for the oils of peanut, rapeseed, soybean, niger, sesame, and sunflower were similar, and the average was 45.2×10^{-5}. The ratio for linseed oil was 38.4×10^{-5}, and for coconut fat it was similar to that of milk fat.

INTRODUCTION

The degree of unsaturation of a fat can be expressed in terms of iodine value or iodine number as determined by the standard Wijs method (1,2). Iodine is absorbed quantitatively, under the conditions of the method, by unsaturated fatty acids or triglycerides at the point of unsaturation. In the Wijs method, considerable care is required in the standardization of Wijs reagent, weighing of sample, and also in the titrations involved in the method in order to get reproducible results.

It was observed that iodinated milk fat obtained after it was reacted with Wijs reagent showed a change in refractive index as compared to the refractive index of the original milk fat. The change in refractive index was observed to have a very good correlation with the iodine number of the milk fat. Therefore, based on the differences in refractive indices of milk fat before and after iodination by Wijs reagent, a simple and precise method involving the use of a refractometer has been suggested in this paper for the estimation of degree of unsaturation of milk fat. The usefulness of the method for the estimation of iodine numbers of vegetable fats has also been indicated.

MATERIALS AND METHODS

Milk Fat

Milk fat samples, 18 each of cow (Nos. 1-18) and buffalo (Nos. 19-36), were prepared from milks of individual animals kept at the farm of the Institute. Cream was separated from the milk. The cream was heated to ca. 110 C till fat separated and then filtered through paper (Whatman No. 4) to obtain clear fat. Six samples of milk fat of buffalo (Nos. 37-42), produced in cotton tract areas where the animals are largely fed on cotton seeds, were procured from commercial sources.

Vegetable Fats

Samples of peanut, sesame, niger, rapeseed, sunflower, and coconut oils were obtained from commercial sources. The oils of soybean and linseed were prepared in the laboratory by hexane extraction.

Determination of Iodine Numbers

Iodine number of fat was determined by the Wijs method according to AOCS (1) The Wijs reagent was prepared by passing dry chlorine gas into iodine solution (1.3%) in glacial acetic acid such that the ratio of iodine to chlorine was 1.1 ± 1. In the method, appropriate amounts of fat dissolved in carbon tetrachloride were reacted with excess Wijs reagent, and the excess Wijs reagent was back titrated against standard sodium thiosulphate solution to determine the absorbed iodine. The average of duplicate values, which did not differ by more than 0.4 units of iodine number in the cases of milk fat and coconut fat and 1.0 unit of iodine number in the cases of the other vegetable oils, was taken.

After the determination, the titration mixture was allowed to stand in a separating funnel. The carbon tetrachloride portion of the mixture which contained the iodinated fat was separated, dried over anhydrous sodium sulphate, and the solvent evaporated to get the iodinated fat. The refractive index of the fat was measured using an Abbe Refractometer (Carlzeis Jena, Model G) at 40 C in daylight.

The Wijs reagent was also prepared according to Indian Standard (2), which was as follows: Eight grams of iodine trichloride was dissolved in 450 ml glacial acetic acid. Nine grams of iodine was dissolved separately in 450 ml glacial acetic acid. The iodine solution was added gradually to the iodine trichloride solution until the color was changed to a reddish brown. Ca. 40 ml more of iodine solution was added, and the mixture was diluted with acetic acid till 10 ml of the mixture was equivalent to 20 ml of 0.1 N sodium thiosulphate solution. This reagent was also used for iodination of milk fat.

Iodination of Milk Fat under Variable Conditions and Their Effect on Refractive Index of iodinated Fat

Two milliliter aliquots of fat solution in chloroform, containing 0.2 g of fat, were taken in glass stoppered test tubes. Various quantities of Wijs reagent were added such that the excess Wijs reagent added in different tubes was equivalent to 25, 50, 100, and 150% of iodine absorbed by the fat. This was calculated from the iodine number of the samples. After a reaction time of 1 hr, a 5% solution of sodium thiosulphate was added to each of the tubes, with vigorous shaking, till all the iodine color was discharged. Iodinated fat was extracted with ca. 15 ml of chloroform. The extract was dried over anhydrous sodium sulphate, and the solvent was evaporated over a water bath to get iodinated fat. It was finally dried in an oven at 100 C till constant refractive index was obtained.

The fat solution added with optimum amounts of Wijs reagent were kept for various lengths of time from 0.5 to 1.5 hours in order to study the effect of reaction time on the refractive index of iodinated fat.

In another set of experiments similar to the above, various amounts of 2.5% solution of mercuric acetate in glacial acetic acid were added to the reaction mixture to study its catalytic effect on the reaction time for iodination.

Iodination of Standard Unsaturated Lipid Compounds

Authentic compounds (a) oleic acid, (b) methyl oleate, (c) linoleic acid, (d) triolein, (e) trilinolein, and (f) trilinolenin were iodinated, and their refractive indices were measured before and after iodination.

RESULTS AND DISCUSSION

The iodine numbers and the refractive indices of the

TABLE I

Iodine Numbers and Refractive Indices of Milk Fats and Standard Lipids, Before and After Iodination

Sample number	Iodine number	Refractive index (RI)		Change in RI	Ratio[a]	Calculated iodine number[b]
		Iodinated	Original			
1	2	3	4	5	6	7
1	34.0	1.4682	1.4509	0.0173	50.9	34.1
2	31.4	1.4689	1.4529	0.0160	51.0	31.6
3	32.4	1.4686	1.4520	0.0166	51.2	32.7
4	29.3	1.4665	1.4516	0.0149	50.9	29.4
5	29.4	1.4662	1.4513	0.0149	50.7	29.4
6	26.1	1.4643	1.4511	0.0132	50.6	26.0
7	25.3	1.4633	1.4506	0.0127	50.2	25.0
8	31.0	1.4671	1.4513	0.0158	51.0	31.2
9	26.6	1.4638	1.4504	0.0134	50.4	26.4
10	25.1	1.4627	1.4498	0.0129	51.4	25.4
11	23.1	1.4617	1.4502	0.0115	49.8	22.7
12	21.1	1.4609	1.4500	0.0109	51.7	21.5
13	28.2	1.4651	1.4507	0.0144	51.1	28.4
14	28.1	1.4652	1.4510	0.0142	50.5	28.0
15	29.7	1.4666	1.4515	0.0151	50.8	29.9
16	28.7	1.4658	1.4513	0.0145	50.5	28.6
17	31.9	1.4670	1.4510	0.0160	50.2	31.6
18	26.7	1.4643	1.4507	0.0136	50.9	26.8
19	23.5	1.4619	1.4499	0.0120	51.1	23.7
20	24.3	1.4624	1.4502	0.0122	50.2	24.1
21	27.9	1.4645	1.4504	0.0141	50.5	27.8
22	26.4	1.4640	1.4507	0.0133	50.4	26.2
23	28.1	1.4651	1.4507	0.0144	51.2	28.4
24	25.0	1.4627	1.4499	0.0128	51.2	25.2
25	27.7	1.4646	1.4507	0.0139	50.2	27.4
26	25.2	1.4632	1.4504	0.0128	50.8	25.2
27	30.5	1.4669	1.4516	0.0153	50.2	30.2
28	30.6	1.4670	1.4516	0.0154	50.3	30.4
29	25.8	1.4634	1.4503	0.0131	50.8	25.8
30	24.9	1.4629	1.4502	0.0127	51.0	25.0
31	22.8	1.4624	1.4507	0.0117	51.3	23.1
32	27.9	1.4643	1.4504	0.0139	49.8	27.4
33	20.4	1.4607	1.4503	0.0104	51.0	20.5
34	21.8	1.4611	1.4499	0.0112	51.4	22.1
35	24.1	1.4627	1.4504	0.0123	51.0	24.3
36	21.8	1.4607	1.4497	0.0110	50.5	21.7
37	31.5	1.4693	1.4534	0.0159	50.5	31.4
38	33.7	1.4703	1.4534	0.0169	50.1	33.3
39	34.6	1.4704	1.4530	0.0174	50.3	34.3
40	34.5	1.5708	1.4534	0.0174	50.4	34.3
41	31.1	1.4681	1.4523	0.0158	50.8	31.2
42	30.3	1.4679	1.4523	0.0156	51.5	30.8
				Average:	50.7	
Lipid						
Triolein	82.4	1.5032	1.4620	0.0412	50.0	
Trilinolein[c]	172.1	1.5338	1.4652	0.0686	39.9	
Trilinolenin[d]	25.8	1.5575	1.4815	0.0760	29.5	
Methyl oleate	82.4	1.4840	1.4426	0.0414	50.2	
Oleic acid	88.4	1.4900	1.4505	0.0395	44.7	
Linoleic acid	180.7	1.5335	1.4620	0.0715	39.6	

[a]Ratio = change in RI:iodine number x 10^{-5}.

[b]Iodine numbers calculated from the average ratio 50.7.

[c]RI measured at 45 C.

[d]RI measured at 50 C.

milk fat samples and also the refractive indices of the corresponding iodinated fats, isolated from the titration mixtures, were determined, and the values are presented in Table I. The iodine numbers obtained covered a wide range of values from 20.4 to 34.6 (Table I) as the samples chosen were from milks of individual animals of both cow and buffalo and from animals of cotton tract area (Nos. 37 to 42) which show high iodine numbers.

It may be seen from Table I that iodinated fat showed higher refractive index than the original fat. The correlation coefficient (r) between the iodine numbers and the differences between the refractive indices of the iodinated fats and those of the corresponding original fats was 0.9993. This indicated that there was a very high degree of correlation between the iodine number and the change in refractive index due to iodination of the fat. Therefore, change in refractive index of the fat can be used as a basis for calculating iodine number of fat. The average of ratios of change in refractive index to iodine number was 50.7 x 10^{-5} for milk fat. The iodine numbers of milk fats calculated from this ratio are also included in the Table I. It may be seen from the table that the calculated values did not differ from those obtained by Wijs method by more than 0.5 units.

The iodine numbers obtained on triolein, trilinolein, trilinolenin, methyl oleate, oleic acid, and linoleic acid and their refractive indices before and after iodination are also given in Table I. The ratio of change in refractive index to iodine number obtained for each of the substances is also included in the table. The ratios obtained in the cases of triolein and methyl oleate were within the range of ratios obtained in the case of milk fats (Table I).

The ratios obtained in the cases of oleic and linoleic acids are less than those obtained for triolein or the milk

fat. Therefore, presence of free fatty acids in milk fat will tend to give lower iodine values when determined by this method. However, this will be generally very small. Even if the free oleic acid content is 1% of the fat, the lowering of iodine number will be less than 0.08 units.

The effect of adding varying amounts of Wijs reagent to fat (0.2 g) for a reaction time of 1 hr on the change in refractive index due to iodination is indicated in Table II. The effect of adding mercuric acetate as catalyst, as suggested by Hofmann and Green (3), on the change in refractive index in 1 hr is also shown in Table II. It may be seen from the table that an excess of Wijs reagent equivalent to 100-150% of that absorbed by the fat was required to get maximum and constant values of refractive index for iodinated fat. The presence of the catalyst did not affect the values, and the amount of excess of Wijs reagent needed in this case was also the same.

In this iodination reaction, chloroform was used instead of carbon tetrachloride for dissolving fat, as chloroform is more convenient to distill from the extracts. This did not affect refractive index of iodinated fat, as was seen from the values of refractive indices for some of the samples in Tables I and II. The effect of time on the iodination reaction is shown in Table III. Hofmann and Green (3) added mercuric acetate to the Wijs reaction mixture and reduced the reaction time to 3 min. Similarly, in the presence of the catalyst, here, the refractive index of the iodinated fat had reached a maximum and constant value when measured after a reaction time of 3 min. The amount of mercuric acetate needed depended on the unsaturation and the amount of fat taken. For 0.2 g of milk fat, ca. 2 ml of 2.5% mercuric acetate solution in acetic acid was adequate for the purpose, and addition of higher quantities did not affect the values. In the absence of the catalyst, reaction period of 1 hr was needed (Table III).

It was found that final heating of iodinated fat to remove traces of solvent was an important step in getting constant values. After distilling the solvent from the extract of iodinated fat, drying in the oven at 100 C for 15 min was

TABLE II

Effect of Quantity of Wijs Reagent on Refractive Index of Iodinated Milk Fat, With and Without Catalyst

Sample number (0.2 g)	Excess of Wijs reagent (%)	RI of iodinated fat	
		Without catalyst	With catalyst
39	25	1.4681	1.4692
	50	1.4686	1.4698
	100	1.4704	1.4704
	150	1.4704	1.4704
11	50	1.4597	1.4612
	100	1.4617	1.4617
	150	1.4617	1.4617
10	50	1.4609	1.4617
	100	1.4625	1.4627
	150	1.4627	1.4627

TABLE III

Effect of Reaction Time on Refractive Index of Iodinated Milk Fat, With and Without Catalyst

Sample number	Reaction time (min)	RI of iodinated fat[a]	
		1	2
41	30	1.4673	1.4675
	60	1.4680	1.4680
	90	1.4680	1.4680
	3 (with catalyst)	1.4680	1.4680
12	30	1.4597	1.4602
	60	1.4610	1.4610
	90	1.4610	1.4610
	3 (with catalyst)	1.4610	1.4610

[a]Values of duplicate analyses.

TABLE IV

Iodine Numbers and Refractive Indices of Vegetable Fats, Before and After Iodination

Oil	Sample number	Iodine number	Refractive index (RI)		Change in RI	Ratio[a]	Average ratio	Calculated iodine number[b]
			Iodinated	Original				
1	2	3	4	5	6	7	8	9
Peanut	1	92.4	1.5073	1.4648	0.0425	46.0	46.3	94.0
	2	92.8	1.5078	1.4648	0.0430	46.4		95.1
	3	92.5	1.5078	1.4648	0.0430	46.6		95.1
Rapeseed	1	102.1	1.5125	1.4653	0.0472	46.3	46.3	104.4
	2	102.9	1.5130	1.4655	0.0475	46.2		105.1
	3	103.0	1.5132	1.4655	0.0477	46.3		105.5
Soybean	1	124.3	1.5250	1.4678	0.0572	46.0	45.9	126.5
	2	129.8	1.5272	1.4678	0.0594	45.8		131.4
	3	125.3	1.5250	1.4676	0.0574	45.8		127.0
Niger	1	133.5	1.5295	1.4705	0.0590	44.2	44.2	130.5
	2	134.5	1.5297	1.4705	0.0592	44.0		131.0
	3	129.8	1.5277	1.4700	0.0577	44.5		127.7
Sesame	1	111.5	1.5173	1.4678	0.0495	44.4	44.5	109.5
	2	111.9	1.5173	1.4676	0.0497	44.4		109.9
	3	110.0	1.5170	1.4678	0.0492	44.7		108.8
Sunflower	1	119.2	1.5205	1.4680	0.0525	44.1	44.1	116.2
	2	119.4	1.5210	1.4682	0.0528	44.1		116.8
	3	119.9	1.5212	1.4682	0.0530	44.2		117.3
						Average:	45.2	
Coconut	1	8.7	1.4560	1.4516	0.0044	50.6	50.1	8.8
	2	9.8	1.4567	1.4517	0.0050	51.0		10.0
	3	10.7	1.4569	1.4517	0.0052	48.6		10.3
Linseed	1	177.0	1.5378	1.4700	0.0678	38.8	38.4	176.5
	2	175.0	1.5375	1.4700	0.0675	38.5		175.8

[a]Ratio = change in RI:iodine number x 10^{-5}.

[b]Iodine numbers for peanut to sunflower oils were calculated from the average ratio 45.2, and those of coconut and linseed oils were calculated from their average ratios 50.1 and 38.4, respectively.

found adequate. The iodinated fat obtained will be clear and will have only the color of the original fat. Overheating tended to give a red tinge to the iodinated fat. Appearance of a slight red tinge did not affect the values. Interestingly, it was observed that when sufficient excess (100-150% of Wijs reagent was not added for the reaction, the iodinated fats obtained developed intense red tinge either during evaporation of the solvent or during the final drying in the oven. Therefore, this forms a good indication for judging whether sufficient excess of Wijs reagent has been taken or not for the reaction. Traces of solvent can also be removed by exposing the iodinated fat to the atmosphere for 3-5 min after spreading it as a thin film on the prism of the refractometer.

This method has several advantages over the Wijs method. The method is simple and gives highly reproducible results. The method does not involve any weighing of sample or titration which may introduce errors. Accurate standardization of Wijs reagent was not necessary. Wijs reagents aged (at ca. 30 C) even up to 3 months did not affect refractive indices of the iodinated fats. The use of a refractometer gives measurement of considerable precision, and this is yet another advantage of the method.

The method recommended for the determination of iodine number of milk fat by refractometry is as follows: Ca. 0.2 ml of milk fat was pipetted into a glass stoppered test tube, and 2 ml chloroform was added. Ca. 10 ml Wijs reagent was then added, followed by addition of 2 ml of 2.5% mercuric acetate solution. After keeping the mixture for 3 min, 5% solution of sodium thiosulphate was added till all the iodine color was discharged. The iodinated fat was extracted with 15 ml of chloroform. The extract was dried over anhydrous sodium sulphate, and the solvent was evaporated to get iodinated fat. It was finally dried in an oven at 100 C for 15 min.

Application of this method for the determination of iodine numbers of some of the vegetable fats was also studied. The iodine number, refractive indices, before and after iodination, and the corresponding ratio of change in refractive index to iodine number obtained for each of the oil samples is given in Table IV.

The ratio of change in refractive index to iodine number obtained in the case of the reference compound trilinolenin was less than that of the trilinolein, which was less than that of the triolein (Table I) by a constant difference of ca. 10.3×10^{-5}. It appears that the ratios are affected by the number of double bonds in the fatty acids of the triglycerides. This is also evident from the date obtained for the vegetable fats. The ratio obtained in the case of coconut oil was similar to that of milk fat. The ratios obtained in the cases of the other oils were less than that of milk fat and this can be attributed to the presence of much higher amounts of linoleate in these fats than in milk fat (4). The ratios for oils of peanut, rapeseed, soybean, niger, sesame, and sunflower can be considered to be similar, and the average of the ratios was 45.2×10^{-5}. From this ratio, when iodine numbers were calculated for each of the above oils (Table IV), the errors as compared to those obtained by Wijs method did not differ by more than 2.8%. The average ratio obtained in case of linseed oil was lower than those of the other fats. This again can be attributed to the presence of large amounts of linolenate in linseed oil (5). Therefore, a separate ratio has to be used for calculating iodine number of linseed oil.

It appears that average ratios can be worked out for individual or groups of oils to obtain reasonably accurate iodine numbers.

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