International Fishmeal & Oil Manufacturers Association

RING TESTS COMPARING ANALYTICAL TECHNIQUES FOR MEASURING IODINE VALUE (PART 2)

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Introduction

The American Oil Chemists Society (AOCS) methods are widely used for contract purposes in the trade of oils and fats.

The traditional method for determining iodine value (AOCS official method Cd1-25) makes use of the solvent carbon tetrachloride. In a number of countries this solvent is now banned for use in laboratories because of its carcinogenic properties. Consequently this method of analysis has been modified using cyclohexane as a solvent (AOCS recommended practice Cd1b-87).

An earlier ring test using fish oils over the normal range of iodine value (Research Report 1993-6) showed that the iodine values obtained using this modified method were lower than those using the traditional method. It was postulated that this might be due to difficulties in accurately determining the end point of the reaction due to an emulsion formed. It was suggested that this problem might be overcome if cyclohexane was substituted by a 1 : 1 mixture of cyclohexane and glacial acetic acid. The purpose of this new ring test was to compare the traditional method with a method using cyclohexane/acetic acid as the solvent mixture.

Methods

Ten laboratories agreed to participate in this ring test. The names and addresses of the participating laboratories are given in Appendix 1. One laboratory failed to make any returns. Two laboratories performed only one of the specified ring tests and were therefore omitted from the statistical assessment of the results.

Each participating laboratory received from the distribution centre in USA eight samples of oil and were asked to analyse each sample in duplicate (open duplicates). Any additional analyses were also asked to be reported. Each sample consisted of 57 g in a sealed amber glass bottle. Each laboratory was asked to analyse the eight samples using carbon tetrachloride (Cd1-25) and a modification of the method Cd1d-92 in which cyclohexane is replaced by a 1 : 1 mixture of cyclohexane and glacial acetic acid. The time of the reaction with Wijs solution was set for 1.0 hours only. The details of both methods were sent to each laboratory with the protocol (Appendices 2 and 3). The modifications to the officially published method are shown by handwritten corrections in Appendix 3.

Laboratories were asked to keep the samples in a freezer and in the dark before and between analyses. Unknown to recipient laboratories each received only four samples of oil but in hidden duplicates. The oils were the same as those distributed in the previous collaborative trial (Research Report Number 1993-6). The sample distribution

centre obtained eight primary samples of fish oil, four selected to be low in IV (< 150) and four selected to be high in IV (> 150). The low IV oils were described as:

1. Sand eel
2. Herring
3. Capelin
4. Menhaden stearin.

The high IV oils were described as:

5. Mackerel
6. Anchovy
7. Pilchard + menhaden (blend)
8. Menhaden.

The eight primary oils were distributed to participating laboratories (two low and two high IV oils to each) according to a statistical pattern designed to give overall balance to comparisons between the oils. Each pair of laboratories represented a complete set of the eight oils. The design was such that within twelve laboratories every low IV oil was compared with every other low IV oil twice within a laboratory and similarly every high IV oil was compared twice within a laboratory with all other high IV oils. Because only seven laboratories submitted acceptable results the statistical distribution was therefore not complete.

Statistical analysis was carried out in several steps. The data were initially scrutinised for possible gross errors. For each analytical method, the standard deviation for the open duplicate analyses was calculated from the differences between the pairs of values, separately for laboratory and then combined over all laboratories. Using the first reported analysis (A) of the open duplicates, differences between the hidden duplicates were calculated for each laboratory from which the standard deviation within each laboratory and pooled over laboratories was calculated. This analysis was repeated with the second reported open duplicate (B). Using the average of the "A" and "B" determinations, the estimated laboratory mean values, corrected for design imbalance in the samples analysed, were derived from a non-orthogonal analysis of variance using the algorithm GENSTAT (1). The same type of analysis, but employing in turn the "A" and "B" determinations, provided estimates of the "Between Laboratory Standard" Deviation. In evaluating error estimates, separate analyses were deemed necessary because of the lack of independence of the two determinations. The presence of outlier laboratories was tested for using the Cochran test for a laboratory with high within laboratory (hidden) variance and the Grubbs test for extreme deviation of laboratory means, according to IUPAC 1993 (2). In the latter test mean values of the four oils, adjusted for differences in the oils, were used instead of carrying out the test on each material one at a time since not all laboratories analysed the same material. The two methods were compared by computing the differences within laboratory and sample, followed by an analysis of variance of these differences. The "Within Laboratory" variances for the two methods were compared using the Wilcoxon Signed Rank Test (see, for example, LLOYD (3)). The same test was used to examine differences in the errors of determinations of HIGH and LOW oils.

Results and Discussion

As stated in the Methods section, the two laboratories returning incomplete data were omitted from the analysis. For Laboratory 1 Sample 9 the cyclohexane/acetic acid method resulted in A and B values (open duplicates) differing by eight units, but there was an additional replicate which agreed well with the B value and therefore these latter two values were used. Similarly Laboratory 5 Sample 10 using the cyclohexane/acetic acid method showed considerable differences between samples A and B whereas the additional replicate agreed closely with sample B. Therefore these latter two results were also used. No other substitutions were necessary. No laboratories were rejected as outliers in either analytical method.

Table 1 summarises the mean value by each method and the three "error" estimates being the standard deviations of single determinations as estimated from

(a) Open duplicates
(b) Hidden duplicates (repeatability)
(c) Between laboratories (reproducibility).

Table 2 gives more detail and shows the standard deviations for each laboratory and method based on the open duplicates and the hidden duplicates.

Table 3 shows the mean values and the hidden duplicate standard deviations for both methods after separating the samples into the high iodine value samples and the low iodine value samples.

The two methods gave closely similar absolute values both overall and for the low and high IV oils separately. The previous standard method may, therefore, be replaced by the new safer method without any change in absolute value.

As in previous collaborative studies of methods which involve manual determination of colour change in titrimetric end points the open duplicates tended to agree more closely than the hidden duplicates, indicating that the second (B) determination is not completely independent of the initial (A) determination. Table 2 indicates that while the open and hidden standard deviations are similar in some laboratories, other laboratories have 2 to 4 fold higher values for the hidden duplicates. The best estimate of the real within laboratory variability (repeatability) is then given by the standard deviation based on the hidden duplicate. The IUPAC 1993 protocol for collaborative studies prefers only one analysis on each of two hidden or split levels of each material for the estimation of repeatability but will accept estimates based on open duplicates when it is not practical to use the better experimental design. In the present study the hidden duplicate A values represent the ideal IUPAC case and are the preferred values. Similarly, the A values for between laboratory standard deviation (reproducibility) are preferred. The B values for both hidden duplicates and between laboratory variation tend to be smaller, but not significantly so, than the A values. In the statistical sense the B values cannot be assumed to be independent or unbiased but the lower values may also represent improved accuracy when the analyst has already had one preliminary titration to

determine the colour change.

The standard deviations (SD; within laboratory) for the high and low IV oils were similar and thus appear to be independent of the absolute value. Expressed as the Relative Standard Deviation (RSD_r%) or Coefficient of Variation (100SD/mean) the within laboratory variability is a little over 1% for low IV oils and a little under 1% for high IV oils.

Earlier methods using carbon tetrachloride (AOCS method Cd 1-25) and cyclohexane (AOCS Cd 1b-87) specified that oils with IV < 150 should be reacted for one hour and those with IV > 150 for two hours. In the previous collaborative trial (Research Report Number 1993-6) increasing reaction time for high IV oils had no effect in either method. The agreement between the means and SD for the two methods (Cd 1-25 and Cd 1d-92 modified) in the present study for both the low and high IV oils confirms that one hour reaction time is sufficient irrespective of IV value.

Table 4 displays the estimated mean determination for each laboratory, for each of the two analytical methods and corrected for design imbalance in the samples analysed. These means were derived from the entire data-base, that is by analysing the average of the A and B determinations. Table 4 also displays the derived mean difference between the two methods together with the pooled overall difference and its standard error. The laboratory mean values were closely similar and the good agreement between laboratories for both methods is further demonstrated by the between laboratory standard deviations given in Table 1 (A values). These are only a little greater than the within laboratory variation with a coefficient of variation of 1.2% for the standard method and 1.3% for the new cyclohexane-acetic acid method. There is no consistent bias of one method over the other from one laboratory to another and the mean difference (Table 4) is not significantly different from zero.

The results of this collaborative trial confirm those of another collaborative study of the same two methods applied to a range of vegetable and animal oils, including one sample of low IV fish oil (Firestone, 1994) (4) in showing cyclohexane-acetic acid can be used in place of carbon tetrachloride without loss of precision. The repeatability and reproducibility reported in that trial for fish oil were 0.5 and 1.1 respectively for the cyclohexane-acetic acid method compared with 1.55 and 1.98 in the present study.

Conclusion

The previous ring test (Research Report Number 1993-6) reported that replacement of carbon tetrachloride as the solvent with cyclohexane in the analysis of fish oils for iodine value gave a figure which was 2.7 units lower when averaged over all eight primary oils. However, the difference was greater with oils selected as having an IV greater than 150 at 3.8 compared with oils of IV less than 150 where the difference was 1.6. It was postulated that this difference might be due to the difficulty in determining the end point during the colour change in the reaction due to the emulsions formed using cyclohexane. It was further postulated that a mixture of cyclohexane and acetic acid might overcome this difficulty.

The results of this collaborative trial show that this is so and no significant differences were recorded in mean iodine values between the methods for either low or high IV oils or in their repeatability or reproducibility. The similar repeatability of both methods for low and high IV oils confirms previous findings that one hour reaction time is sufficient for high IV oils. Consequently the modified method using cyclohexane and acetic acid and one hour reaction time (Appendix 3) is recommended for fish oil over the usual range of iodine values.

From these results the repeatability and reproducibility figures for this recommended method are 1.55 and 1.98 respectively.

REFERENCES

1. *GENSTAT (1988).* GENSTAT 5 Reference Manual, Clarendon Press, Oxford.

2. *IUPAC (1993).* Protocol for the design, conduct and interpretation of collaborative studies. Revised Lisbon, Portugal, 4 August 1993. Editor William Horwitz, Food and Drug Administration, Washington DC. Interdivisional working party on harmonisation of quality assurance schemes for analytical laboratories. International Union and Pure and Applied Chemistry.

3. *LLOYD, E. (ed). (1984).* Handbook of Applicable Mathematics Vol 6B, New York, Wiley.

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TABLE 1

Table summarising three "Error" estimates, being the standard deviations of single determinations as estimated from;

(a) Open Duplicates,
(b) Hidden Duplicates,
(c) Between Laboratories

The main tabulated figure is the standard deviation, which has also been presented as percentage of the mean value (in brackets).

		Method	
		Carbon Tetrachloride	**AOCS Cd 1d-92 (Mod.)**
Mean Determination		154.9	155.0
Open Duplicate		1.08 (0.70)	0.66 (0.43)
Hidden Duplicate	A*	1.71 (1.10)	1.55 (1.00)
	B*	1.01 (0.65)	1.29 (0.83)
Between Laboratory	A	1.81 (1.17)	1.98 (1.28)
	B	1.43 (0.92)	1.79 (1.15)

*Comparison of hidden duplicate results in the first analysis of the samples (A) and hidden duplicates in second analysis of the same samples (B)

TABLE 2

Table displaying the between "Open Duplicate", and between "Hidden Duplicate" standard deviations for each Laboratory, and method.

Laboratory	**Open Duplicate**		**Hidden Duplicate**			
	Cd 1-25	CD 1d-92 (mod)	Cd 1-25		Cd 1d-92 (mod)	
			A	B	A	B
1	2.02	0.94	3.14	0.87	2.06	1.12
3	0.48	0.47	1.62	1.71	1.86	2.01
5	0.66	1.21	2.07	1.08	2.69	1.59
6	0.28	0.34	0.35	0.21	0.16	0.19
7	0.84	0.43	0.54	1.00	0.45	0.90
8	0.13	0.16	0.94	0.94	0.17	0.37
10	1.61	0.50	1.58	0.58	1.25	1.66

NB: Comparison between laboratories should be avoided because each analysed different samples.

TABLE 3						
Table displaying the mean values and the "Hidden" Duplicate standard deviations, by method and by High/Low Sample.						
Sa.	**Cd 1-25**			**Cd 1d-92 (Mod.)**		
	Mean	A SD (%)	B SD (%)	Mean	A SD (%)	B SD (%)
Low	129.8	1.89 (1.46)	0.89 (0.69)	130.0	1.53 (1.18)	1.21 (0.93)
High	180.0	1.53 (0.85)	1.11 (0.62)	180.1	1.56 (0.87)	1.35 (0.75)

TABLE 4			
Table displaying the means and "Method" effect for each laboratory			
Laboratory	**Cd 1-25**	**Cd 1d-92 (Mod)**	**Mean Difference [Cd 1-25 - Cd 1d-92 (Mod)]**
1	155.43	154.22	1.21
3	156.02	155.74	0.28
5	154.59	154.87	-0.28
6	154.64	155.61	-0.97
7	154.00	154.29	-0.29
8	154.70	153.56	1.15
10	154.88	156.86	-1.98
Overall			-0.13/+0.16

COLLABORATING LABORATORIES

Professor J P H Wessels
Fishing Industry Research Institute
Lower Hope Street 15
Rosebank 7700
Cape Province
SOUTH AFRICA

Tel No: +27 21 6899341
Fax No: +27 21 6866116

Mr A P Bimbo
Zapata Haynie Corporation
P O Box 175
Reedville, Virginia 22539
U.S.A.

Tel No: +1 804 453 4211
Fax No: +1 804 453 4722

Dr Domenico Grieco
Associazione Granaria di Milano
Laboratorio Chimico
E Microscopia
Via Isonzo 20
20089 Rozzano MI
Milan, ITALY

Tel No: +39 2 89202095
Fax No: +39 2 57500391

Dr Bjarne Bøe
Fiskeridirektoratet
Sentrallaboratoriet
Strandgaten 229
P O Box 185
5002 Bergen
NORWAY

Tel No: +47 55 23 8000
Fax No: +47 55 23 8090

Mr Stacy Lewis
Cargill Analytical Services
15305 Minnetonka Boulevard
Minnetonka, MN 55345-1512
U.S.A.

Tel No: +1 612 930 0032
Fax No: +1 612 930 0943

Dr S Kmieciak, Technical Director
Polcargo Central Chemical Laboratory, Ltd.
ul Indyjska 13
81-969 Gdynia
POLAND

Tel No: +48 58 204011
Fax No: +48 58 219810

Chemical Laboratory Dr P Wiertz
Dip Chem K Eggert,
Dr U Jorissen Gmbh
Stenzelring 14b
21107 Hamburg
GERMANY

Tel No: +49 40 75 27 090
Fax No: +49 40 75 27 09 35

Mr A Bellido
International Analytical Services S.A.
Av La Marina 3035
San Miguel
Lima 32
PERU

Tel No: +51 14 516680
Fax No: +51 14 641964

SGS Controll-Co MBM
Labor Hamburg
Behringstrasse 154
2000 Hamburg 50
GERMANY

Tel No: +49 40 ~~88 300220~~ 301010
Fax No: +49 40 ~~88 11375~~ 32633

Mr Arne Brodin
SSF
Kjerreidviken 16
5033 Fyllingsdalen
Bergen
NORWAY

Tel No: +47 55 12 3100
Fax No: +47 55 12 3488

A.O.C.S. Official Method Cd 1-25 (corrected 1991)
Revised for IFOMA Ring Test on Fish Oils

Iodine Value of Fish Oils

Wijs Method

Definition: The iodine value is a measure of the unsaturation of fats and oils and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample (% iodine absorbed).
Scope: Applicable to fish oils which do not contain conjugated double bonds (see Notes 1).

Apparatus:

1. Glass stoppered iodine flask, 500 mL.

2. Glass stoppered volumetric flask, conforming to National Bureau of Standards (NBS) tolerances and accurately calibrated to contain 1,000 mL.

3. Pipet, 20 mL.

4. Two pipets, 25mL.
   Note - One 25 mL pipet is reserved for use with the standard potassium dichromate solution. This pipet must conform to NBS tolerances and be accurately calibrated to deliver 25 mL.

5. Bottles, borosilicate, actinic glass, with glass stoppers, 1,000 mL.

6. Filter paper, Whatman No. 41H, or equivalent.

Reagents:

1. Glacial acetic acid, ACS grade. The permanganate test should be applied to be sure that this specification is met.

(a) Permanganate test - Dilute 2 mL of the acid with 10 mL of distilled water and add 0.1 mL of 0.1 N $KMnO_4$. The pink colour must not be entirely discharged within 2 hours.

2. Potassium iodide (KI), reagent grade.

3. Chlorine, 99.8% (see Notes, Caution). Satisfactory commercial grades are available in cylinders, but the gas must be dried by passing through sulfuric acid (sp. gr. 1.84) before introducing into the iodine solution.
   Note - Prepared Wijs (see Notes, Caution and 2) solution is available commercially, and may be used in place of the laboratory prepared solution.

4. Soluble starch, tested for sensitivity. Make a paste with 1 g of starch and a small amount of cold distilled water. Add, while stirring, 200 mL of boiling water. Place 5 mL of this solution in 100 mL of water and add 0.05 mL of 0.1 N iodine solution. The deep blue colour produced must be discharged by 0.05 mL of 0.1 N sodium thiosulfate.

5. Potassium dichromate, reagent grade, is finely ground and dried to constant weight at about 110°C before using.
   Note - A standard sample of potassium dichromate with a certificate of analysis may be obtained from the National Bureau of Standards in Washington, D.C. This sample, or equivalent, is strongly recommended for the primary standard for this method. Treat as directed in the certificate of

analysis accompanying the sample.

6. Sodium thiosulfate ($Na_2S_2O_3.5H_2O$), reagent grade.

7. Iodine, reagent grade.

8. Carbon tetrachloride, reagent grade (see Notes, Caution and Recommendations).

9. Hydrochloric acid (HCl), reagent grade, concentrated, sp. gr. 1.19 (see Notes, Caution).

10. Sulfuric acid (H_2SO_4), reagent grade, concentrated, sp. gr. 1.84 (see Notes, Caution).

11. Iodine monochloride, reagent grade.

Solutions:

1. Potassium iodide (KI) solution, prepared by dissolving 150 g KI in distilled water and making up to 1 litre.

2. Starch indicator solution, prepared and tested as noted in Reagents, 4. Salicylic acid (1.25 g per litre) may be added to preserve the indicator. If long storage is required, the solution must be kept in a refrigerator at 4 to 10°C (40 to 50°F). Fresh indicator must be prepared when the endpoint of the titration from blue to colourless fails to be sharp. If stored under refrigeration, the starch solution should be stable for about two to three weeks.

3. Sodium thiosulfate ($Na_2S_2O_3.5H_2O$) solution, 0.1 N, accurately standardised vs. potassium dichromate primary standard as follows -

(a) Sodium thiosulfate solution 0.1 N, prepared by dissolving 24.9 g of sodium thiosulfate in distilled water and diluting to 1 litre.

(b) The potassium dichromate primary standard should be finely ground, dried at 105°C for 2 hours and cooled in a desiccator. Accurately weigh 0.16 to 0.22 g ± 0.0001 g of potassium dichromate into a 500 ml flask or bottle by difference from a weighing bottle. Dissolve in 25 mL of water, add 5 mL of concentrated hydrochloric acid, 20 mL of potassium iodide solution and rotate to mix. Allow to stand for 5 minutes and then add 100 mL of distilled water. Titrate with sodium thiosulfate solution, shaking continuously until yellow colour has *almost* disappeared. Add 1 to 2 mL of starch indicator and continue the titration, adding the thiosulfate solution slowly until the blue colour just disappears. The strength of the sodium thiosulfate solution is expressed in terms of its normality.

$$\text{Normality of } Na_2S_2O_3 \text{ solution} = \frac{20.394 \times \text{Wt. of } K_2Cr_2O_7}{\text{mL of sodium thiosulfate}}$$

4. Wijs solution (see Notes, Caution and 2), laboratory preparation -
Note - Prepared Wijs solution is available commercially, and may be used in place of the laboratory prepared solution.
Dissolve 13.0 g of iodine in 1 litre of glacial acetic acid. Gentle heat may be necessary to promote solution. Cool, remove a small quantity (100-200 mL) and set aside in a cool place for future use. Pass dry chlorine gas into the iodine solution until the original titration is not quite double. A characteristic colour change takes place in the Wijs solution when the desired amount of chlorine has been added. This may be used to assist in judging the endpoint. A convenient procedure is to add a small excess of chlorine and immediately bring back to the desired titration by addition of some of the original iodine solution which was taken out at the beginning. The original solution and finished Wijs solution are both titrated with $Na_2S_2O_3$ solution as directed in Procedure, 6 and 7. The Wijs solution may be prepared from commercial iodine monochloride as follows -

(a) Stock Solution - Add 317±0.1 g of iodine monochloride to 1 litre of glacial acetic acid and filter through Whatman No. 41H filter paper or equivalent into a clean and dry actinic glass bottle. Filter rapidly to prevent contamination with moisture and store in a cool place. Discard the solution if a precipitate forms on standing.

(b) Wijs Solution - Using a graduate pour 117.0 ± 0.1 mL of the stock solution into a standard 5 pound bottle of glacial acetic acid, and mix well by shaking.

5. The I/Cl ratio of the Wijs solution should be within the limits of 1.10 ± 0.1. The procedure for determining the ratio is as follows -

Iodine Content -

(a) Pour 150 mL of saturated chlorine water into a 500 mL Erlenmeyer flask and add a few glass beads.

(b) Pipette 5 mL of the Wijs solution into the flask containing the saturated chlorine water. Shake, and heat to boiling.

(c) Boil briskly for 10 minutes, cool, and add 30 mL of 2% sulfuric acid and 15 mL of 15% potassium iodide solution.

(d) Mix well, and titrate immediately with 0.1 N sodium thiosulfate solution to a starch endpoint.

Total Halogen Content -

(a) Pour 150 mL of recently boiled distilled water into a clean, dry 500 mL Erlenmeyer flask.

(b) Add 15 mL of 15% potassium iodide solution.

(c) Pipette 20 mL of Wijs solution into the flask, and mix well.

(d) Titrate with 0.1 N sodium thiosulfate solution to a starch endpoint.

Calculation of Halogen Ratio

$$\text{Halogen Ratio } R = \frac{2A}{3B - 2A}$$

A = Titration of iodine content as mL sodium thiosulfate.

B = Titration of total halogen content as mL sodium thiosulfate.

Procedure:

1. Melt the sample, if it is not already liquid (the temperature during melting and filtering should not exceed the melting point of the sample by more than 10°C), and filter through filter paper to remove any solid impurities and the last traces of moisture. The sample must be absolutely dry.
Note - All glassware must be absolutely clean and completely dry.

2. Immediately weigh the sample accurately either directly into a 500 mL flask placed on the balance or into a small weighing bottle which is placed inside the flask. Add 20 mL of carbon tetrachloride (see Notes, Caution) or other solvent (see Notes, Recommendations). The weight of sample must be such that there will be an excess of Wijs solution of 100 to 150% over the amount absorbed. Table 1 is a guide to the size of sample to weigh.

3. Dispense 25 mL of Wijs solution into flask containing the sample, stopper the flask, and swirl to insure an intimate mixture. Immediately set the time for 1.0 hr or 2.0 hrs and store the flasks in the dark at 25 ± 5°C. (see Notes, 3)

4. Prepare and conduct at least two blank determinations with each group of samples simultaneously and similar in all respects to the samples.

5. Remove the flasks from storage and add 20 mL of KI solution, followed by 100 mL of distilled water.

Table 1. Sample weights.

Iodine value		Sample weight		Weighing accuracy
		150% excess	100% excess	
		g	g	g, ±
Less than	3	10	10	0.001
	3	8.4613	10.5760	0.005
	5	5.0770	6.3460	0.0005
	10	2.5384	3.1730	0.0002
	20	0.8461	1.5865	0.0002
	40	0.6346	0.7935	0.0002
	60	0.4321	0.5288	0.0002
	80	0.3173	0.3966	0.0001
	100	0.2538	0.3173	0.0001
	120	0.2115	0.2644	0.0001
	140	0.1813	0.2266	0.0001
	160	0.1587	0.1938	0.0001
	180	0.1410	0.1762	0.0001
	200	0.1269	0.1586	0.0001

6. Titrate with 0.1 N $Na_2S_2O_3$ solution, adding it gradually and with constant and vigorous shaking (see Notes, 4). Continue the titration until the yellow colour has almost disappeared. Add 1 to 2 mL of starch indicator solution and continue the titration until the blue colour has just disappeared.

Calculations:

$$\text{The iodine value} = \frac{(B-S) \times N \times 12.69}{\text{Weight of sample}}$$

Where -
B = Titration of blank
S = Titration of sample
N = Normality of $Na_2S_2O_3$

Notes:

Caution
Carbon tetrachloride is a known carcinogen. It is toxic by ingestion, inhalation and skin absorption. It is a narcotic. It should not be used to extinguish fires. It decomposes to phosgene gas at high temperature. The TLV is 5 ppm in air. A fume hood should be used at all times when using carbon tetrachloride.

Chlorine is a poisonous gas. The TLV is 1 ppm in air. It is a strong oxidising agent and should not be allowed to come in contact with organic materials, hydrogen, powdered metals and reducing agents. A fume hood should be used at all times when using chlorine.

Wijs solution causes severe burns and vapours can cause lung and eye damage. Use of a fume hood is recommended. Wijs solution without carbon tetrachloride is available commercially.

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is an oxidising agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive spattering. Always add the acid to water, never the reverse.

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalation and a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

Recommendations
The most satisfactory replacement found to date for carbon tetrachloride has been cyclohexane [see A.O.C.S. Recommended Practice Cd 1b-87 Revised 1990].

Numbered Notes

1. When the iodine value is determined on materials having conjugated systems, the result is not a measure of total unsaturation, but rather is an empirical value indicative of the amount of unsaturation present. Reproducible results are obtained which afford a comparison of total unsaturation. When the iodine value is required on fatty acids, the preparation and separation are performed as directed in AOCS Official Method Cd 6-38.

2. All Wijs solutions are sensitive to temperature, moisture, and light. Store in a cool and dark place and never allow to come to a temperature above 25-30°C.

3. The indicated reaction times reflect those used in IUPAC Method 2.205, "IUPAC Standard Methods

for the Analysis of Oils, Fats and Derivatives", 7th Edition (1987).

4. Mechanical stirring is very satisfactory for agitating during the addition of thiosulfate.

Changes from AOCS Official Method Cd 1-25 Corrected 1991

1. Title - "Fish oils" for "Fats and oils".
2. Scope - "Fish oils" for "fats and oils".
3. Reagents, item 1 - "Glacial acetic acid".
4. Reagents, item 5 - "Treat as directed in the certificate ....."
5. Procedure, item 2 - Alternative use of weighing bottle and add carbon tetrachloride after weighing.
6. Procedure, item 3 is changed to omit reference to different times for different IV oils and to add specific instructions for storage of reaction flasks.
7. Table 1 is changed for IV ≥ 80.
8. Numbered Notes, item 3, has been changed to omit reference to tung oil and to omit reference to earlier procedures.

AOCS Recommended Practice Cd 1d-92

Iodine Value of Fats and Oils

Cyclohexane–Acetic Acid Method

Definition: The iodine value is a measure of the unsaturation of fats and oils and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample (% iodine absorbed).

Scope: Applicable to all normal fats and oils that do not contain conjugated double bonds (see Notes, 1).

Apparatus

1. Glass-stoppered iodine flasks—500 mL.
2. Glass-stoppered volumetric flasks—1000 mL, for preparing standard solutions.
3. Pipet—25 mL, for accurately dispensing 25.0 mL of Wijs solution.
4. Volumetric dispenser—20 mL, 1-mL adjustability, for 10% potassium iodide (KI) solution.
5. Volumetric dispenser—2 mL, 1-mL adjustability, for starch solution.
6. Volumetric dispenser—50 mL, 1-mL adjustability, for distilled water.
7. Repeater pipet—with filling flask, 20 mL, for cyclohexane.
8. Analytical balance—accurate to ±0.0001 g.
9. Magnetic stirrer.
10. Filter paper—Whatman no. 41H, or equivalent.
11. Beakers—50 mL.
12. ~~Hot air oven.~~
13. Timer.

Reagents

1. Wijs solution—see Notes, Caution and 2.
2. Potassium iodide (KI) solution—100 g/L (10% solution), prepared by dissolving 100 g of reagent grade KI in 1000 mL of deionized water.
3. Cyclohexane—reagent grade (see Notes, Caution and 3).
4. Glacial acetic acid—reagent grade (see Notes, Caution).
5. Reagent for diluting sample—prepared by mixing cyclohexane and glacial acetic acid, 1:1, v/v. The absence of oxidizable matter in the reagent is verified by shaking 10 mL of the reagent with 1 mL of saturated aqueous potassium dichromate solution and 2 mL of concentrated sulfuric acid; no green coloration should appear.
6. Hydrochloric acid—reagent grade (see Notes, Caution).
7. Soluble starch solution—recently prepared, tested for sensitivity (see Notes, 4). Make a paste with 1 g of natural, soluble starch and a small amount of cold distilled water. Add, while stirring, to 100 mL of boiling water. Test for sensitivity by adding 5 mL of the starch solution to 100 mL of water; add 0.05 mL of 0.1 N iodine solution. The deep blue color produced must be discharged by 0.05 mL of 0.1 N sodium thiosulfate.
8. Potassium dichromate—reagent grade. The potassium dichromate is finely ground and dried to constant weight at about 110 C before using.
9. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)—0.1 N, accurately standardized, prepared from reagent-grade $Na_2S_2O_3 \cdot 5H_2O$ (see Notes, 5).

   Standardization of sodium thiosulfate—Weigh 0.16–0.22 g of finely ground and dried potassium dichromate into a 500-mL flask or bottle by difference from a weighing bottle. Dissolve in 25 mL of water, add 5 mL of concentrated hydrochloric acid, 20 mL of potassium iodine solution and rotate to mix. Allow to stand for 5 min, and then add 100 mL of distilled water. Titrate with sodium thiosulfate solution, shaking continuously until the yellow color has *almost* disappeared. Add 1–2 mL of starch indicator and continue the titration, adding the thiosulfate solution slowly until the blue color just disappears. The strength of the sodium thiosulfate solution is expressed in terms of its normality.

$$\text{Normality of } Na_2S_2O_3 \text{ solution} = \frac{20.394 \times \text{wt of } K_2Cr_2O_7}{\text{mL of sodium thiosulfate}}$$

Procedure

1. Melt the sample, if it is not already liquid (the temperature during melting should not exceed the melting point of the sample by more than 10 C), and filter through two pieces of filter paper to remove any solid impurities and the last traces of moisture. ~~The filtration may be performed in an air oven at 100 C, but should be completed within 5 min ± 30 sec.~~ The sample must be absolutely dry.

   Note—All glassware must be absolutely clean and completely dry.

2. ~~After filtration, allow the filtered sample to achieve a temperature of 68–71 ± 1 C before weighing the sample.~~
3. ~~Once the sample has achieved a temperature of 68–71 ± 1 C,~~ immediately weigh the sample into a 500-mL iodine flask, using the weights and weighing accuracy noted in Table 1 (see Notes, 6).
4. Add 15 mL of cyclohexane + acetic acid (Reagents, 5) on top of the sample and swirl to ensure that the sample is completely dissolved.
5. Dispense 25 mL of Wijs solution using the pipet (Apparatus, 3) into the flask containing the sample, stopper the flask and swirl to ensure an intimate mixture. Immediately set the timer for 1.0 ~~or 2.0 hr, depending on the iodine value of the sample: IV < 150, 1.0 hr; IV ≥ 150, 2.0 hr~~ hr (see Notes, 7).

Iodine Value of Fats and Oils

Table 1
Sample weights.

Replace by Table 1 of Cd 1-25

Iodine value expected	Weight, g, ±~~0.001~~
<5	3.000
5–20	1.000
21–50	0.400
51–100	0.200
101–150	0.130
151–200	0.100

6. Immediately store the flasks in the dark for the required reaction time at a temperature of 25 ± 5 C.
7. Remove the flasks from storage and add 20 mL of KI solution, followed by 150 mL of distilled water (see Notes, 8 and 9).
8. Titrate with 0.1 N $Na_2S_2O_3$ solution, adding it gradually and with constant and vigorous shaking (see Notes, 10). Continue the titration until the yellow color has *almost* disappeared. Add 1–2 mL of starch indicator solution and continue the titration until the blue color just disappears.
9. Prepare and conduct at least ~~one~~ two blank determination with each group of samples simultaneously and similar in all respects to the sample.

Calculations

1. The iodine value = $\frac{(B - S) \times N \times 12.69}{\text{wt of sample}}$

Where—

B = titration of blank
S = titration of sample
N = normality of $Na_2S_2O_3$ solution

Precision

1. International Union of Pure and Applied Chemistry (IUPAC)/International Organization for Standardization (ISO) collaborative studies gave the statistical results shown in Tables 2 and 3.

Table 2
Statistical analysis of results obtained in the first IUPAC/ISO collaborative study (1989).[a]

Sample	Mean value		r		R	
	CTC[b]	CHX	CTC	CHX	CTC	CHX
sunflower seed oil	133.6	132.9	3.0	3.6	7.2	4.8
palm oil, refined	53.1	53.0	0.65	0.82	1.43	1.90
fish oil, crude	109.1	108.5	1.67	1.40	4.35	2.80
tung oil	164.5	163.1	3.47	2.39	5.27	4.32
tallow (beef fat)	47.2	46.9	1.37	1.33	2.75	3.10
soybean oil, hydrogenated	63.3	65.3	1.86	13.8	17.2	14.9
palm oil, crude	52.5	52.6	1.29	2.15	2.19	2.89
frying oil, used	37.7	37.7	0.97	1.38	1.18	2.48
fish oil, hydrogenated	63.3	68.6	3.61	1.69	17.2	11.0
palm kernel oil	18.2	18.3	0.17	0.14	0.51	0.64
olive oil	82.3	82.2	0.75	1.75	1.86	2.62

[a]Eleven laboratories participated, comparing mean values and values for r and R (repeatability and reproducibility limits, respectively). Except for hydrogenated oils, the type of solvent used for the determination has little or no influence on the precision of the determination (References, 2).
[b]Solvents used: CTC, carbon tetrachloride; CHX, cyclohexane + glacial acetic acid (1:1).

Table 3
Statistical analysis of results obtained in the second IUPAC/ISO collaborative study (1990).[a]

Sample	Mean value		r		R	
	CTC[b]	CHX	CTC	CHX	CTC	CHX
soybean oil 1, hydrogenated	102.6	102.3	1.4	2.2	4.8	5.1
soybean oil 2, hydrogenated	74.7	74.8	1.7	1.5	3.7	2.1
fish oil, hydrogenated	73.0	72.8	1.7	1.6	2.5	2.3

[a]Seventeen laboratories participated, comparing mean values and values for r and R (repeatability and reproducibility limits, respectively) (References, 2).
[b]Solvents used: CTC, carbon tetrachloride; CHX, cyclohexane + glacial acetic acid (1:1).

Notes

Caution

Wijs solution causes severe burns, and the vapors can cause lung and eye damage. Use of a fume hood is recommended. Wijs solution without carbon tetrachloride is available commercially.

Cyclohexane is flammable and a dangerous fire risk. It is moderately toxic by inhalation and skin contact. The TLV in air is 300 ppm.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalation and is a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

Numbered Notes

1. When the iodine value is determined on materials having conjugated systems, the result is not a measure of total unsaturation, but rather is an empirical value indicative of the amount of unsaturation present.

Iodine Value of Fats and Oils

Reproducible results are obtained which afford a comparison of total unsaturation. When the iodine value is required on fatty acids, the preparation and separation are performed as directed in AOCS Official Method Cd 6-38.

2. Because the preparation of the Wijs solution is time-consuming and involves the use of both hazardous and toxic chemicals, this solution may be purchased from a chemical supplier. Solutions are available which contain no carbon tetrachloride, and such solutions should be used. All Wijs solutions are sensitive to temperature, moisture and light. Store in a cool and dark place, and never allow to come to a temperature above 25–30 C. The laboratory preparation of Wijs solution is noted in AOCS Official Method Cd 1-25.
3. Fresh cyclohexane should be used. Erratic results may be obtained if old cyclohexane is used. See Reagents, 5 for test for presence of oxidizable substances.
4. 1% starch solution may be purchased from a chemical supplier.
5. The sodium thiosulfate solution may be purchased from a chemical supplier. However, it still must be accurately standardized in the laboratory.
6. The weight of the sample must be such that there will be an excess of Wijs solution of 50–60% of the amount added; i.e., 100–150% of the amount absorbed.
7. The indicated reaction times are those specified in the IUPAC Iodine Value Method 2.205 (References, 1) and were the reaction times used in the IUPAC/ISO validation study of the cyclohexane + acetic acid method. Previous AOCS versions of iodine value methods specified a reaction time of 0.5 hr, regardless of the iodine value, but noted that "a longer reaction time may be necessary for oils with high iodine value." The longer reaction times appear to be particularly critical when cyclohexane is used as a replacement for chloroform (References, 3).
8. If the reaction is not terminated within 3 min after the reaction time, the sample must be discarded.
9. The sample must be titrated within 30 min of reaction termination, after which the analysis is invalid.
10. Mechanical stirring is recommended for agitation during the addition of thiosulfate.

References

1. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 2.205.
2. IUPAC collaborative study results using cyclohexane + glacial acetic acid (1:1) appear in *Pure Appl. Chem. 62*:2339 (1990) and were reported in *INFORM 1*:484 (1990).
3. AOCS collaborative study results using cyclohexane alone appear in *J. Am. Oil Chem. Soc. 65*:745 (1988).