41.1.15

AOAC Official Method 993.20
Iodine Value of Fats and Oils
Wijs (Cyclohexane–Acetic Acid Solvent) Method
First Action 1993
Final Action 1996

IUPAC–AOCS–AOAC Method
(Applicable to determination of iodine value for fats and oils which do not contain conjugated double bonds.)

Caution: Wijs solution causes severe burns; vapors can cause lung and eye damage. Use of fume hood is recommended. See Appendix B, Laboratory Safety for procedures on safe handling of acids and organic solvents (cyclohexane).

See Table 993.20A for the results of the interlaboratory study supporting acceptance of the method.

A. Principle
Fat or oil is mixed with iodine monochloride solution to halogenate double bonds in fat or oil. Excess iodine monochloride is reduced to free iodine in presence of potassium iodide, and free iodine is measured by titration with sodium thiosulfate using starch as indicator.

Iodine value (IV), calculated as cg iodine absorbed per g of sample (% iodine absorbed), is a measure of unsaturation of fats and oils.

B. Apparatus
(a) Glass-stoppered iodine flasks.—500 mL.
(b) Glass-stoppered volumetric flasks.—1000 mL, for preparing standard solutions.
(c) Volumetric dispensers.—(1) 25 mL, for Wijs and 15% potassium iodide (KI) solutions. (2) 2 mL, for starch solution. (3) 50 mL, for H₂O.
(d) Repeater pipet.—20 mL, with filling flask, for cyclohexane.
(e) Analytical balance.—Accurate to ±0.0001 g.
(f) Filters.—Ashless, coarse grade (Whatman No. 541 is suitable).
(g) Hot air oven.—Capable of maintaining 100°C within ±1.5°C.

C. Reagents
(a) Potassium iodide (KI) solution.—15%. Dissolve 15 g KI in 100 mL H₂O.
(b) Wijs iodine solution.—(1) Dissolve 13 g resublimed I in 1 L acetic acid, and pass in dried (through H₂SO₄) Cl until original NaS₂O₃ titration of solution is not quite doubled. (Characteristic color change at end point indicates proper amount of Cl. Convenient method is to reserve some of original I solution, add slight excess of Cl to bulk of solution, and bring to desired titer by readdictions of reserved portion.) Or: (2) Dissolve 16.5 g ICl in 1 L acetic acid.

Determine I/Cl ratio as follows:
(1) Iodine content.—Pipe 5 mL Wijs solution into 500 mL Erlenmeyer flask containing 150 mL saturated Cl–H₂O and some glass beads. Shake, heat to boiling, and boil briskly 10 min. Cool, add 30 mL H₂SO₄ solution (1 + 49) and 15 mL 15% KI solution, and titrate immediately with 0.1M Na₂S₂O₃.

\[ \frac{X}{3B - 2X} \]

where \( X = mL 0.1 M NaS₂O₃ \) required for I content and \( B = mL \) required for total halogen content. If I/Cl ratio is not 1.10 ± 0.1, add I or Cl to correct ratio.

Standardized Wijs solution may be obtained from commercial suppliers (specify without carbon tetrachloride).

| Table 993.20A Interlaboratory study results for determination of iodine value by Wijs method using carbon tetrachloride solvent or cyclohexane–acetic acid (1 + 1) solvent |
|---------------------------------|----------------|---------------|----------------|---------------|----------------|
| Sample                        | CTC a          | CHX b         | s₁             | s₂             | RSD₁, %        | RSD₂, %        |
| Sunflower                      | 133.6          | 132.9         | 1.4            | 1.7            | 2.6           | 1.7           |
| Refined palm                   | 53.1           | 53.0          | 0.1            | 0.2            | 0.3           | 0.5           |
| Crude fish                     | 109.1          | 108.5         | 0.7            | 0.5            | 0.6           | 0.5           |
| Tung                           | 164.5          | 163.1         | 2.0            | 1.4            | 2.5           | 1.2           |
| Tallow (beef)                  | 47.2           | 46.9          | 0.2            | 0.2            | 0.5           | 0.5           |
| Crude palm                     | 52.5           | 52.6          | 0.3            | 0.4            | 0.4           | 0.5           |
| Used frying                    | 37.7           | 37.7          | 0.1            | 0.2            | 0.4           | 0.5           |
| Palm kernel                    | 18.2           | 18.3          | 0.01           | 0.01           | 0.1           | 0.1           |
| Olive                          | 82.3           | 82.2          | 0.2            | 0.5            | 0.6           | 0.6           |
| HSBO-1                         | 102.6          | 102.3         | 0.5            | 0.8            | 1.8           | 1.9           |
| HSBO-2                         | 74.7           | 74.8          | 0.5            | 0.4            | 1.0           | 0.6           |
| HFO d                          | 73.0           | 72.8          | 0.4            | 0.4            | 0.7           | 0.6           |

a Carbon tetrachloride.

b Cyclohexane–acetic acid (1 + 1).

c Hydrogenated soybean oil.

d Hydrogenated fish oil.

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Store in amber bottle sealed with paraffin until ready for use. Wijs solutions are sensitive to temperature, moisture, and light. Store in dark at 30°C.

(c) Soluble starch solution.—Mix paste of 1 g starch with small amount cold H2O. While stirring, add 200 mL boiling H2O. Test for sensitivity: place 5 mL starch solution in 100 mL H2O and add 0.05 mL 0.1M iodine solution; deep blue color produced must be discharged by 0.05 mL 0.1M sodium thiosulfate solution. (Note: 1% starch solution, commercially available, is suitable.)

(d) Potassium dichromate (K2Cr2O7).—Finely grind and dry to constant weight (ca 110°C) before using in D.

(e) Sodium thiosulfate (Na2S2O3·5H2O) solution.—0.1M. Standardize as in D.

(f) Acids.—(1) Hydrochloric acid (HCl).—Concentrated, sp gr 1.19. (2) Acetic acid (C2H4O2).—Glacial. (3) Sulfuric acid (H2SO4).—Concentrated.

(g) Cyclohexane.—[Note: Erratic results may result if cyclohexane is old, i.e., contains oxidizable matter; see (h).]

(h) Cyclohexane–acetic acid solvent.—Mix cyclohexane, (g), and acetic acid, (f)(2), 1 + 1 (v/v). Verify absence of oxidizable matter in solvent by shaking 10 mL solvent with 1 mL saturated aqueous K2Cr2O7 solution and 1 mL H2SO4, (f)(3). No green color should appear.

D. Standardization of Sodium Thiosulfate Solution

Accurately weigh 0.16–0.22 g dried, finely ground K2Cr2O7, C(d), to nearest 0.0001 g into 500 mL flask, dissolve in 25 mL H2O, add 5 mL HCl, C(f)(1), and 20 mL KI solution, C(b), and rotate to mix. Let stand 5 min. Add 100 mL H2O. Titrate with sodium thiosulfate solution, C(e), shaking continuously until yellow color has almost disappeared. Add 1–2 mL starch indicator solution, C(c), and continue adding thiosulfate solution slowly until blue color just disappears.

\[
Na_2S_2O_3 \text{ solution molarity, } M = \frac{20.394 \times \text{wt } K_2Cr_2O_7}{E \times \text{mL sodium thiosulfate}}
\]

E. Determination

Melt test sample, if not already liquid (do not exceed sample melting point by >10°C). Pass test sample through double layer of filter paper to remove any solid impurities and traces of H2O (filtration may be performed in air oven, ca 100°C, but should be completed within 5 min ±30 s). Test sample must be absolutely dry. (Note: All glassware must be absolutely clean and completely dry.)

Let filtered test sample cool to 68–71°C. Immediately weigh amount of test sample indicated in Table 993.20B into clean, dry 500 mL flask, B(a).

Prepare at least 2 blank determinations to run with each sample group.

Add 15 mL cyclohexane–acetic acid solvent, C(h), to each test sample and swirl to ensure that it is completely dissolved.

Dispense 25 mL Wijs solution into flask containing test sample, stopper flask, and swirl to mix. Immediately set timer for 1.0 or 2.0 h, depending on iodine value of sample (IV <150, 1.0 h; IV ≥150, 2.0 h) and store flasks in dark at 25°–5°C for duration of reaction.

Remove flasks from dark, add 20 mL KI solution, C(b), and mix. Add 150 mL H2O and gradually titrate with 0.1M standard Na2S2O3 solution, D, with constant and vigorous shaking or mechanical stirring. Continue titrating until yellow color has almost disappeared. Add 1–2 mL starch indicator solution to flasks and continue titrating until blue color has just disappeared. (Note: If reaction is not terminated by addition of KI and H2O within 3 min past 1.0 or 2.0 h reaction time, sample must be discarded. The sample must be titrated within 30 min of reaction termination; if not, the analysis is invalid.)

F. Calculation

\[
\text{Iodine value (IV)} = \frac{(B - S) \times M \times 12.69}{\text{wt of fat or oil}}
\]

where \(B\) = titration of blank (mL); \(S\) = titration of test sample (mL); \(M\) = molarity of Na2S2O3 solution.


Revised: March 1997

Table 993.20B Test sample weights

<table>
<thead>
<tr>
<th>Value</th>
<th>Test sample, g</th>
<th>Accuracy, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>10.58–8.46</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>3.17–2.54</td>
<td>0.2</td>
</tr>
<tr>
<td>20</td>
<td>1.59–1.27</td>
<td>0.2</td>
</tr>
<tr>
<td>40</td>
<td>0.79–0.63</td>
<td>0.2</td>
</tr>
<tr>
<td>80</td>
<td>0.40–0.32</td>
<td>0.2</td>
</tr>
<tr>
<td>120</td>
<td>0.26–0.21</td>
<td>0.2</td>
</tr>
<tr>
<td>160</td>
<td>0.20–0.16</td>
<td>0.2</td>
</tr>
<tr>
<td>200</td>
<td>0.16–0.13</td>
<td>0.2</td>
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