Start sampler. After last cup has been sampled, let system continue until steady baseline is obtained.

(3) Shut-down.—After each series of analyses, pump methanol–water solution (ca 80 + 20 v/v) through LC column until baseline is stable.

F. Calculation

Plot peak area vs concentration of *p*-TSA standard solutions. Determine *p*-TSA concentrations (C_x) of test solutions by interpolation. To calculate *p*-TSA concentration in samples, multiply C_x by 5 (dilution factor). Report results to nearest 0.01 mg *p*-TSA/1 kg sample.

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FOOD COMPOSITION AND ADDITIVES

Determination of the Iodine Value of Oils and Fats: Summary of Collaborative Study

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Two collaborative studies were conducted using the Wijs method for determining the iodine value in a wide range of vegetable and animal oils and fats. The results obtained when using carbon tetrachloride were compared to those obtained when using a substitute solvent mixture of cyclohexane and glacial acetic acid. The values reported for the iodine values indicate that the cyclohexane and acetic acid mixture can be used in place of carbon tetrachloride without loss of precision. The method has been adopted first action by AOAC INTERNATIONAL as an IUPAC/AOCS/AOAC method.

Eleven laboratories from 9 countries participated in a collaborative study conducted in 1989. The test samples represented a range of lipid materials, including 7 vegetable oils consisting of olive oil, refined palm kernel oil, crude and refined palm oil, tung oil, sunflower seed oil, and hydrogenated soybean oil; 3 animal fats consisting of crude and hydrogenated fish oil; and tallow. Each of the 11 materials was provided as blind duplicates. The participants were required to determine the iodine value once only using carbon tetrachloride, and once only using a mixture of cyclohexane and glacial acetic acid (1 + 1).

Eighteen laboratories from 11 countries participated in a second collaborative study conducted in 1990 (1). The test samples included 3 materials in blind duplicates (hydrogenated soybean oils at 2 levels of hydrogenation, and hydrogenated fish oil). Solvents used in the second study were the same as those used in the 1989 study.

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The recommendation was approved by the Committee on Food Nutrition and was adopted by the Official Methods Board of the Association. See "Changes in Official Methods of Analysis" (1994) *J. AOAC Int.* **77**, Jan/Feb issue, and "Official Methods Board Actions" (1993) *The Referee*, **17**, July issue.

D. Firestone is the AOAC INTERNATIONAL General Referee on Fats and Oils.

993.20 Iodine Value of Fats and Oils—Wijs (Cyclohexane–Acetic Acid Solvent) Method

IUPAC/AOCS/AOAC Method

First Action 1993

(Applicable to determination of iodine value for fats and oils that do not contain conjugated double bonds.)

Method Performance:

See Table **993.20A** for method performance data.

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

A. Principle

Fat or oil sample 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.

Table 993.20A Method performance for determination of iodine value by wijs method using carbon tetrachloride solvent or cyclohexane–acetic acid (1 + 1) solvent

	Mean value		s_r		s_R		RSD_r, %		RSD_R, %	
Sample	CTC[a]	CHX[b]	CTC	CHX	CTC	CHX	CTC	CHX	CTC	CHX
Sunflower	133.6	132.9	1.4	1.7	3.4	2.3	1.1	1.3	2.6	1.7
Refined palm	53.1	53.0	0.1	0.2	0.3	0.4	0.2	0.3	0.5	0.7
Crude fish	109.1	108.5	0.7	0.5	1.7	1.1	0.6	0.5	1.6	1.0
Tung	164.5	163.1	2.0	1.4	3.1	2.5	1.2	0.9	1.9	1.5
Tallow (beef)	47.2	46.9	0.2	0.2	0.5	0.4	0.5	0.5	1.0	0.8
Crude palm	52.5	52.6	0.3	0.4	0.4	0.5	0.5	0.8	0.8	1.0
Used frying	37.7	37.7	0.1	0.2	0.2	0.3	0.4	0.5	0.4	0.9
Palm kernel	18.2	18.3	0.01	0.01	0.03	0.04	0.1	0.1	0.2	0.2
Olive	82.3	82.2	0.2	0.5	0.6	0.8	0.3	0.6	0.7	0.9
HSBO[c]–1	102.6	102.3	0.5	0.8	1.8	1.9	0.5	0.8	1.7	1.8
HSBO–2	74.7	74.8	0.5	0.4	1.0	0.6	0.6	0.5	1.3	0.8
HFO[d]	73.0	72.8	0.4	0.4	0.7	0.6	0.6	0.6	0.9	0.8

[a] Carbon tetrachloride.
[b] Cyclohexane–acetic acid (1 + 1).
[c] Hydrogenated soybean oil.
[d] Hydrogenated fish oil.

Iodine value (IV), calculated as centigrams (cg) iodine absorbed per g 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 H_2O.

(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° within ±1.5°.

C. Reagents

(a) *Potassium iodide (KI) solution.*—15%. Dissolve 15 g KI in 100 mL H_2O.

(b) *Wijs iodine solution.*—*(1)* Dissolve 13 g resublimed I in 1 L acetic acid, and pass in dried (through H_2SO_4) Cl until original $Na_2S_2O_3$ 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 readditions of reserved portion.) Or: *(2)* Dissolve 16.5 g ICl in 1 L acetic acid.

Determine I/Cl ratio as follows:

Iodine content.—Pipet 5 mL Wijs solution into 500 mL erlenmeyer flask containing 150 mL saturated Cl–H_2O and some glass beads. Shake, heat to boiling, and boil briskly 10 min. Cool, add 30 mL H_2SO_4 solution (1 + 49) and 15 mL 15% KI solution, and titrate immediately with 0.1N $Na_2S_2O_3$.

Total halogen content.—Pipet 20 mL Wijs solution into 500 mL erlenmeyer flask containing 150 mL recently boiled and cooled H_2O and 15 mL 15% KI solution. Titrate immediately with 0.1N $Na_2S_2O_3$.

$$\frac{I}{Cl} = \frac{2X}{3B - 2X}$$

where X = mL 0.1N $Na_2S_2O_3$ 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).

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) *Soluble starch solution.*—Mix paste of 1 g starch with small amount cold H_2O. While stirring, add 200 mL boiling H_2O. Test for sensitivity: place 5 mL starch solution in 100 mL H_2O and add 0.05 mL 0.1N iodine solution; deep blue color produced must be discharged by 0.05 mL 0.1N sodium thiosulfate solution. (*Note*: 1% starch solution, commercially available, is suitable.)

(d) *Potassium dichromate ($K_2Cr_2O_7$).*—Finely grind and dry to constant weight (ca 110°) before using in *D*.

(e) *Sodium thiosulfate ($Na_2S_2O_35H_2O$) solution.*—0.1N. Standardize as in *D*.

(f) *Acids.*—*(1) Hydrochloric acid (HCl).*—Concentrated, sp gr 1.19. *(2) Acetic acid ($C_2H_4O_2$).*—Glacial. *(3) Sulfuric acid (H_2SO_4).*—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 $K_2Cr_2O_7$ solution and 1 mL H_2SO_4, (**f**)*(3)*. No green color should appear.

D. Standardization of Sodium Thiosulfate Solution

Accurately weigh 0.16–0.22 g dried, finely ground $K_2Cr_2O_7$, *C*(**d**), to nearest 0.0001 g into 500 mL flask, dissolve in 25 mL H_2O, 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 H_2O. 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.

$$\text{Na}_2\text{S}_2\text{O}_3 \text{ solution normality, N} = \frac{20.394 \times \text{Wt } K_2Cr_2O_7}{\text{mL sodium thiosulfate}}$$

E. Determination

Melt test sample, if not already liquid (do not exceed sample melting point by >10°). Pass test sample through double layer of filter paper to remove any solid impurities and traces of H_2O (filtration may be performed in air oven, ca 100°, but should be completed within 5 ± 0.5 min). Sample must be absolutely dry. (*Note*: All glassware must be absolutely clean and completely dry.)

Let filtered test sample cool to 68–71°. 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 sample 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° for duration of reaction.

Table 993.20B Sample Weights

I value	Sample, g	Accuracy, mg
3	10.58–8.46	± 0.5
10	3.17–2.54	± 0.2
20	1.59–1.27	± 0.2
40	0.79–0.63	± 0.2
80	0.40–0.32	± 0.2
120	0.26–0.21	± 0.2
160	0.20–0.16	± 0.2
200	0.16–0.13	± 0.2

Remove flasks from dark, add 20 mL KI solution, *C*(**b**), and mix. Add 150 mL H_2O and gradually titrate with 0.1N standard $Na_2S_2O_3$ 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 H_2O 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

$$\text{Iodine value (IV)} = \frac{(B - S) \times N \times 12.69}{\text{Wt of sample}}$$

where B = titration of blank (mL); S = titration of sample (mL); N = normality of $Na_2S_2O_3$ solution.

Ref.: *Pure & Appl. Chem.* **62**, 2339(1990); JAOAC **77**, May/June 1994

Reference

(1) Pocklington, W.D. (1990) *Pure Appl. Chem.* **62**, 2340–2343