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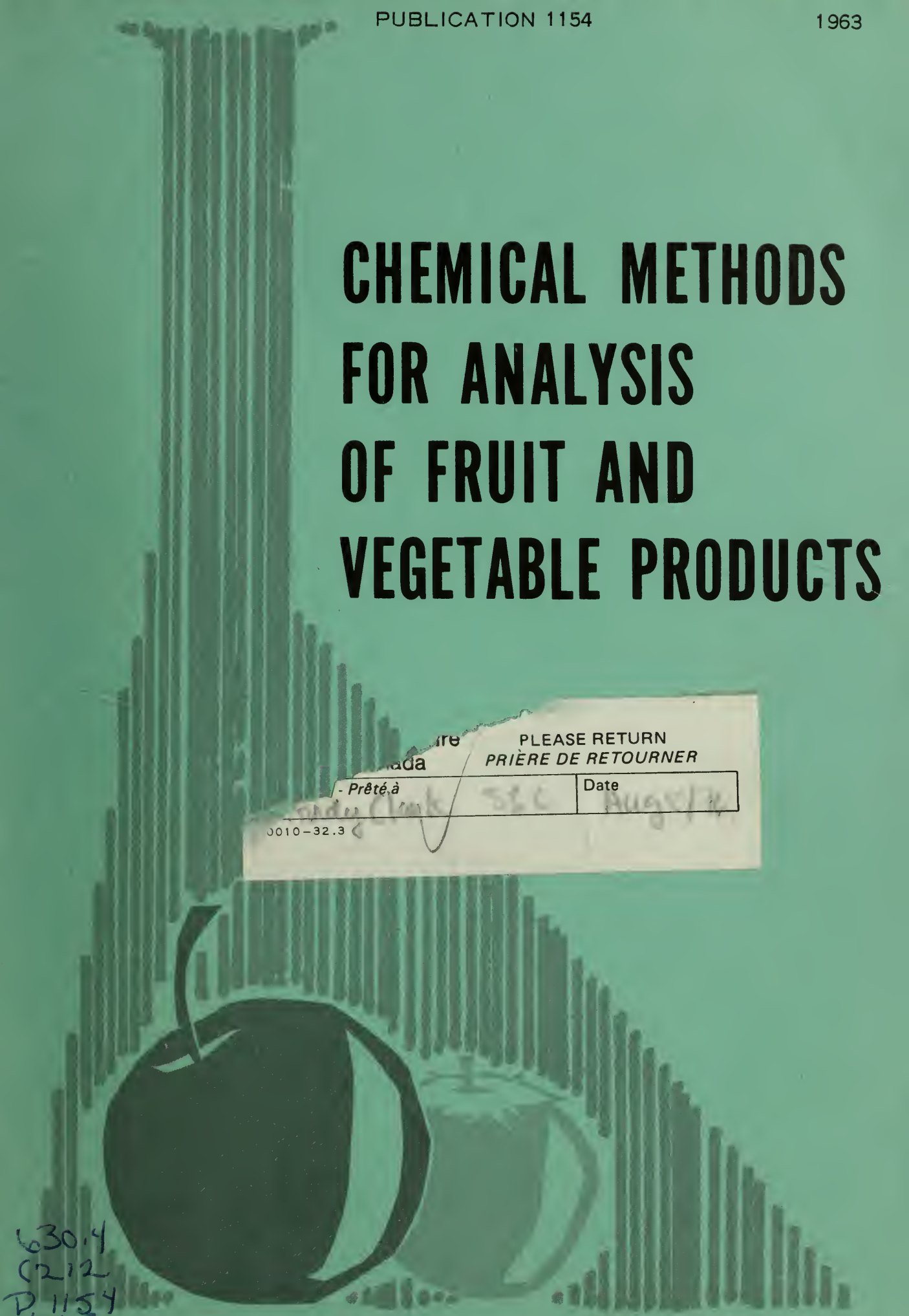

CANADA DEPARTMENT OF AGRICULTURE

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CHEMICAL METHODS FOR ANALYSIS OF FRUIT AND VEGETABLE PRODUCTS

Compiled

by

J. A. Ruck

Contribution No. B7, Research Station, Summerland, B.C.

Research Branch

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CONTENTS

INTRODUCTION

In the Fruit and Vegetable Processing Laboratory, Research Station, Summerland, the work includes research with new products, analyses of commercial fruit products, and the development of procedures for use in establishing grades and standards. In addition, special problems in the fruit and vegetable processing industry are studied.

Chemical methods of analysis are constantly changing through revision, and adoption of improved techniques. The methods described in this publication are those most commonly used for analyzing the products listed and are in current use at the Summerland laboratory. These methods should be useful for all units and laboratories engaged in fruit and vegetable research.

Since the printing of the first edition of this manual, several new methods have been adopted and the procedures are included in this edition.

After the outline of each procedure is a list of references that constitutes acknowledgment of information used in this publication.

CHEMICALS

All chemicals mentioned in the following procedures are of chemically pure (C.P.) grade or equivalent quality. Reagents commonly used, such as mineral acids and ammonia, are not included in the lists of reagents for individual procedures. When they are mentioned in procedures, C.P. concentrated reagents are to be used.

Concentrations of solid reagent solutions are indicated in percentages on a weight/volume basis: a definite weight of solid reagent is dissolved in water and made up to a total volume of 100 ml. Concentrations of liquid reagents are indicated in percentages on a volume/volume basis: the given volume of the concentrated reagent is diluted to a total volume of 100 ml. with water unless otherwise stated. Reagents are sometimes designated on a ratio basis such as (4 + 1), which means 4 parts of reagent to 1 part water. Water mentioned in any procedure is distilled water.

Technical grade chemicals usually cost appreciably less than C.P. chemicals and where possible they should be used. In the following procedures these are indicated by the abbreviation (tech.) after the reagent.

APPARATUS AND EQUIPMENT

Special apparatus and equipment required for the various procedures are given in the description of the analytical method involved. Unless otherwise stated, temperatures mentioned are in degrees Centigrade. When liquid measurements of solutions and reagents are required to be accurate, pipette measurements are specified. Otherwise, a graduated cylinder or similar measure is satisfactory.

CALCULATION OF RESULTS

The results of analysis of food products are usually expressed on the basis of fresh weight, but occasionally on a moisture-free basis. The values in the following procedures are expressed as percentages by weight or percentages by volume of the original products.

The weights and aliquots suggested in the procedures are usually sufficient where an average amount of the constituent is present. In some cases a more suitable weight or aliquot of the sample may have to be determined by experiment.

JAMS, JELLIES AND MARMALADES

PREPARING THE MATERIAL

Remove 1/3 to 1/2 of the material for analysis from the container and blend for 1 to 2 minutes in a Waring blendor. Take the portion vertically through the container to avoid removing excess berries or seeds that may have floated to the top. Stir the portion well with a spoon before taking each sample for weighing.

WATER-INSOLUBLE SOLIDS

Principle

A weighed sample is boiled with water to extract soluble material. The insoluble material is collected on a previously dried, weighed filter paper, and washed with hot water. The insoluble solids are then dried in an oven and weighed.

Procedure

Weigh to the nearest 0.01 gm., on a torsion balance, duplicate 25-gm. samples of the blended portion. Transfer each duplicate to a 400-ml. beaker with hot water and dilute with additional hot water to about 200 ml. Mix and boil gently for 15 to 20 minutes. Transfer one of the duplicate samples to a 250-ml. volumetric flask, cool and make up to volume at 20°. (The filtrate from this is used later for determining acidity. Mark filtrate "A.") Filter separately the sample from the volumetric flask and that from the beaker through No. 4 Whatman paper (15.0 cm.) that was previously washed with hot water, oven-dried for 2 hours at 100°, cooled in a desiccator and weighed in a covered weighing dish (7-cm. diam.). Wash with 800 ml. of hot water, loosening the water-insoluble solids from filter paper with each addition. Transfer filter paper to original weighing dish. Dry overnight at 100 to 105°, cool in desiccator and weigh.

Calculations

% water-insoluble solids = wt. of dry insoluble material × 4.

TOTAL ACIDITY

Principle

Total acidity is determined by titrating an aliquot of the water extract with standard sodium hydroxide to pH 8.1 using a pH meter.

Procedure

Use filtrate A from the previous water-insoluble solids determination. Pipette 50 ml. of the filtrate into 250-ml. beaker. Add about 100 ml. water and titrate with 0.1N NaOH to pH 8.1, using a pH meter. Record the amount of NaOH required and calculate total acidity. Total acid is expressed as the percentage of the predominant acid in the fruit. In small fruits such as strawberries, raspberries, black currants, gooseberries and citrus fruits, this is taken as citric acid; in plums, cherries, peaches and apricots, as malic acid; and in grapes, as tartaric acid.

Calculations

Assume the titration required 5.7 ml. 0.1N NaOH and the original sample weight was 25 gm. The sample was diluted to 250 ml. and 50-ml. aliquot used for titration:

$$\% \text{ total acid} = \frac{1}{10} \times \frac{\text{equiv. wt. of acid} \times \text{normality of NaOH} \times \text{titer}}{\text{wt. of sample}}$$

$$= \frac{1}{10} \times \frac{70.0 \times 0.10 \times 5.7}{5}$$

$$= 0.140 \times 5.7$$

Equivalent wt. of acids:

Citric (monohydrate)	= 70.0 gm.	Tartaric	= 75.0 gm.
Malic	= 67.0 gm.	Acetic	= 60.0 gm.

SULFUR DIOXIDE

(Official Method)

Principle

The sample is acidified and the evolved sulfur dioxide is swept with carbon dioxide or nitrogen into cold hydrogen peroxide, where the sulfurous acid is oxidized to sulfuric acid. The latter is determined by titration with standard sodium hydroxide.

Reagents

1. Hydrogen peroxide—3% solution

   Dilute 200 ml. 30% hydrogen peroxide to about 1,400 ml. in a 2,000-ml. graduate. Mix by pouring solution back and forth from a 2,000-ml. beaker to the graduate. Take 100-ml. portion of diluted solution (100-ml. graduate) and titrate in a 250-ml. beaker on pH meter with 0.1N NaOH to pH 4.1. Do not return this portion to main solution. Calculate amount of NaOH required to neutralize the main solution; add this amount, stir, check the pH.

Standardization of H_2O_2 solution

Pipette 10 ml. of the solution into a 100-ml. volumetric flask and make up to volume at 20°. Pipette 5 ml. of this diluted solution into 500-ml. flask, add about 300 ml. water and 10 ml. 6N H_2SO_4 and titrate with 0.1N potassium permanganate to first permanent pink color. If an exact 3% solution is required, calculate as follows:

1 ml. 0.1N $KMnO_4$ = 0.0017 gm. H_2O_2

If the titration required 9.1 ml. 0.105N $KMnO_4$ then

$\%\ H_2O_2 = 0.017 \times 9.1 \times$ normality of $KMnO_4 \times$ dilution

$= 0.017 \times 9.1 \times 0.105 \times 200$

Adjust solution to 3.0% with water and *always* store in a refrigerator.

2. Bromophenol blue—0.1% solution

   Dissolve 0.1 gm. in 100 ml. water.

3. Phenolphthalein indicator—0.1%

   Dissolve 0.1 gm. in 50 ml. ethyl alcohol and dilute to 100 ml. with water.

4. Sodium carbonate—saturated solution

   Dissolve enough sodium carbonate (tech.) to prepare a saturated solution. Add several drops of phenolphthalein. Discard this solution when it becomes decolorized by adsorption of acids in the carbon dioxide.

Apparatus

The apparatus illustrated in Figure 1 is pyrex throughout. All parts have either 24/40 standard taper or 18/9 spherical joints.

Procedure

Circulate cold water through condenser. Add from a graduated cylinder 20 ml. 3% hydrogen peroxide to the Erlenmeyer flask and 5 ml. to the trap. Assemble and connect to condenser. Weigh 50 gm. of blended portion and rinse into 500-ml. flask through gas inlet tube, using 300 ml. water. Replace gas inlet tube immediately, making sure all connections are well greased and tight. Remove the gas inlet tube, and slowly add 20 ml. conc. HCl. Replace the tube and see if bubbles enter the receiving flasks. If not, check joints for leaks. Adjust CO_2 (passed through a gas washing bottle filled with sodium carbonate solution) or nitrogen (99.9% pure) to give a flow of 15 to 20 bubbles per minute through the tube. Connect the 500-watt heater and turn to the high position. In about 5 minutes, when solution starts to boil, adjust heater to give a slow boil. Dried fruits or vegetables require 1 hour's boiling. Thirty minutes is enough for all other products. After the solution boils for the required time, wash the hydrogen peroxide solution from the trap into the Erlenmeyer flask. Rinse the trap with water.

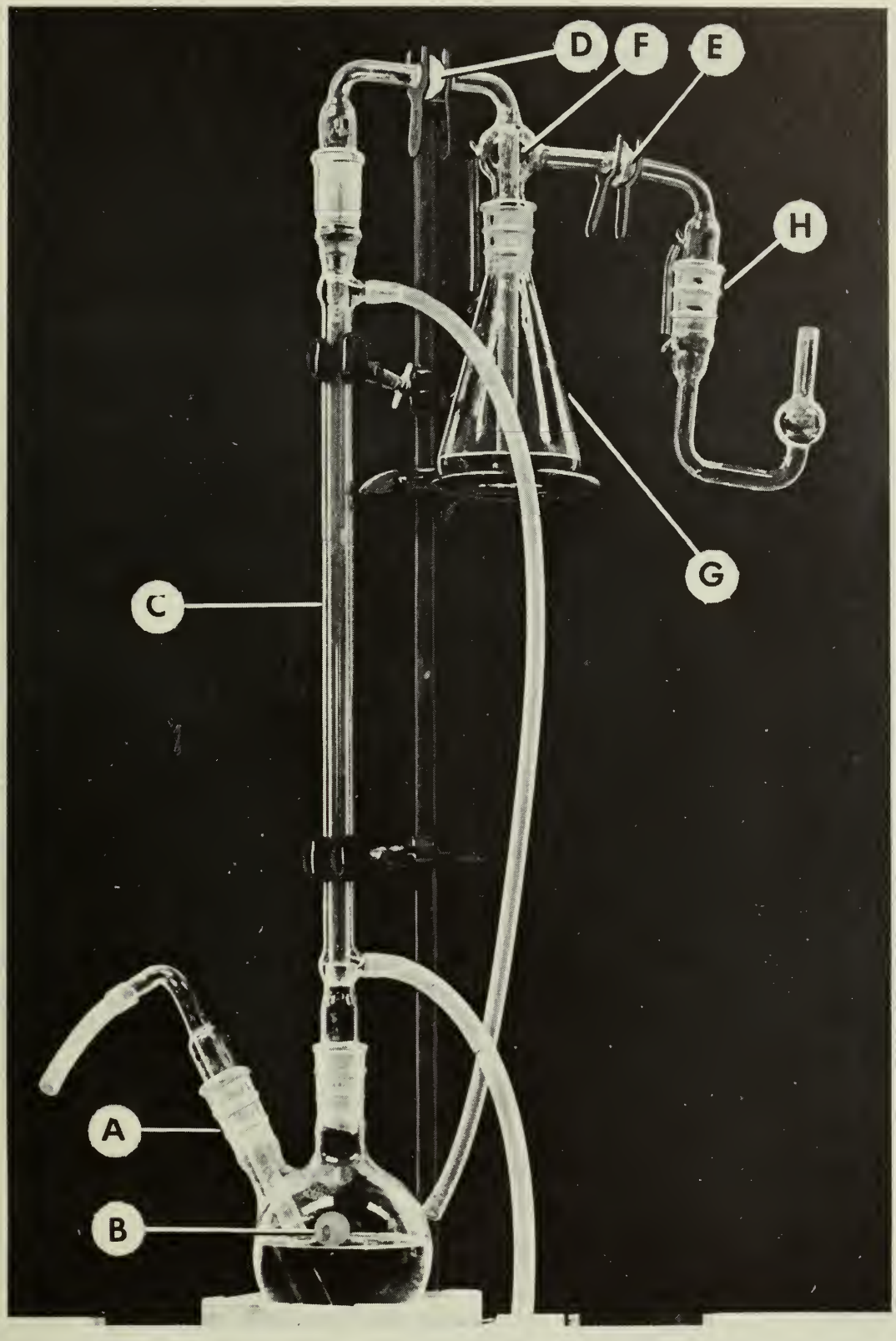

Figure 1.—All-glass apparatus for determining sulfur dioxide. A, Gas inlet tube. B, 500-ml. round-bottom flask. C, Condenser, 400 mm. jacket length. D-E, 18/9 spherical joints. F, Special adapter. G, 250-ml. Erlenmeyer flask. H, Trap.

Add 3 drops of bromophenol blue indicator and titrate with 0.05N NaOH solution to pale sky blue end point, using a 5-ml. microburette (for samples very high in SO_2 use a 50-ml. burette and 0.1N NaOH). Run a blank titration on 20 ml. of hydrogen peroxide and correct the results accordingly.

Calculations

1 ml. 0.1N NaOH = 3.2 mg. SO_2

If 50-gm. sample required 4.1 ml. 0.05N NaOH:

$$\text{p.p.m. } SO_2 = \frac{32 \times 1000 \times \text{normality of NaOH} \times \text{ml. of NaOH}}{\text{wt. of sample}}$$

$$= \frac{32 \times 1000 \times 0.05 \times 4.1}{50}$$

References

Association of Official Agricultural Chemists. Official methods of analysis. 8th ed. pp. 507-509. Washington, D.C. 1955.

Shipton, J. Estimation of sulfur dioxide in dried foods. Food Preservation Quarterly 14 (3): 54-56. 1954.

SOLUBLE SOLIDS

Principle

Soluble solids are determined with a refractometer equipped with a percent sugar scale.

Procedure

Take representative samples of a well-mixed portion of jam or jelly free from seed and fiber, place on refractometer prisms and read directly at 20°. If temperature correction is necessary, use the correction factor given in Table 1. It is preferable to have the instrument maintained at constant temperature by circulating water through the prisms. For very accurate results a correction should be made for water-insoluble solids as follows:

$$\% \text{ soluble solids} = \% \text{ solids by refractometer} \times \frac{(100-a)}{100}$$

where a = % water-insoluble solids.

pH

Principle

The effective acidity is determined by taking a direct reading on a pH meter.

Procedure

Standardize the pH meter with a pH 4.0 buffer solution.

Place 50 to 75 gm. of well-mixed portion in a 100-ml. beaker and read on pH meter. When the first reading is completed, wipe electrodes with small piece of cotton soaked in distilled water. Rinse electrodes with water from wash bottle, dry with a piece of filter paper and continue with the next determination.

Table 1. – Temperature Corrections for the Standard Model of Sugar Refractometer Calibrated for 20° C.[1]

Temperature	Percentage of dry substance													
	5	10	15	20	25	30	35	40	45	50	55	60	65	70
	Subtract from dry-substance percentages													
15° C.	.29	.31	.33	.34	.34	.35	.36	.37	.37	.38	.39	.39	.40	.40
16	.24	.25	.26	.27	.28	.28	.29	.30	.30	.30	.31	.31	.32	.32
17	.18	.19	.20	.21	.21	.21	.22	.22	.23	.23	.23	.23	.24	.24
18	.13	.13	.14	.14	.14	.14	.15	.15	.15	.15	.16	.16	.16	.16
19	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08	.08	.08	.08	.08
	Add to dry-substance percentages													
21	.07	.07	.07	.07	.08	.08	.08	.08	.08	.08	.08	.08	.08	.08
22	.13	.14	.14	.15	.15	.15	.15	.15	.16	.16	.16	.16	.16	.16
23	.20	.21	.22	.22	.23	.23	.23	.23	.24	.24	.24	.24	.24	.24
24	.27	.28	.29	.30	.30	.31	.31	.31	.31	.31	.32	.32	.32	.32
25	.35	.36	.37	.38	.38	.39	.40	.40	.40	.40	.40	.40	.40	.40
26	.42	.43	.44	.45	.46	.47	.48	.48	.48	.48	.48	.48	.48	.48
27	.50	.52	.53	.54	.55	.55	.56	.56	.56	.56	.56	.56	.56	.56
28	.57	.60	.61	.63	.63	.64	.64	.64	.64	.64	.64	.64	.64	.64
29	.66	.68	.69	.71	.72	.73	.73	.73	.73	.73	.73	.73	.73	.73
30	.74	.77	.78	.79	.80	.80	.81	.81	.81	.81	.81	.81	.81	.81

[1]Proceedings of the Ninth Session of the International Commission for Uniform Methods of Sugar Analysis, London. 1936.

ARTIFICIAL FOOD DYES

(Water-Soluble Coal Tar Dyes)

Principle

The dye is absorbed by a piece of white woolen cloth from an acidified solution. Individual pieces of the cloth are moistened separately with conc. HCl, conc. H_2SO_4, conc. NH_4OH and 10% NaOH. The colors developed are compared with those of known dyes.

This method is not conclusive but does provide a useful and quick estimation of the presence of some artificial food dyes, particularly where the dye is present in pure form.

Procedure

Dilute 20- to 40-gm. sample with 1 to 3 volumes of water, add 3 or 4 drops of conc. HCl and a small piece of white woolen cloth (Nun's veiling). Heat to boiling and then cool. Rinse the dyed cloth thoroughly in running water, squeeze out excess water and cut into four small pieces; place each in a separate depression of a white porcelain spot plate. Moisten separate pieces with conc. HCl, conc. H_2SO_4, 10% NaOH and conc. NH_4OH.

The hue of many coloring matters varies markedly upon treatment with acids or alkalies. This variation is also influenced by concentration of reagents and quantity of dye present. An unknown dye color should be compared with that of a known dye at about the same visual density. Table 2 shows color changes produced on wool dyed with 0.1 to 0.5% solutions of permitted food dyes.

Table 2.—Color Reactions Produced on Dyed Fibers by Various Reagents

Coloring matter	Hydrochloric acid	Sulfuric acid	10% NaOH solution	Ammonium hydroxide
Amaranth	Slightly darker	Violet to brownish	Dull brownish to orange-red	Little change
Erythrosine	Orange-yellow	Orange-yellow	No change	No change
Ponceau 3R	Little change	Little change	Dull orange	Little change
Ponceau SX	Deeper red	Deeper red	Orange-yellow	Orange-yellow
Tartrazine	Slightly darker	Slightly darker	Little change	Little change
Naphthol yellow S	Almost decolorized	Very pale, dull brown	No change	No change
Light green SF yellowish	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
Brilliant blue FCF	Yellow	Yellow	No change	No change

References

Association of Official Agricultural Chemists. Official methods of analysis, 7th ed. p. 658. Washington, D.C. 1950.

PECTIN

(As Calcium Pectate)[1]

Principle

Pectin is precipitated as calcium pectate from an acid solution by the addition of calcium chloride. The calcium pectate precipitate is washed with water until chloride-free, then dried and weighed.

[1] Modification Carré and Haynes method by Dr. McInney, Food and Drugs Laboratory, Ottawa, 1944.

Reagents

1. Acetic acid—normal solution (approximate)
   Dilute 30 ml. C.P. glacial acetic acid to 500 ml. with water.
2. Calcium chloride—normal solution (approximate)
   Dissolve 27.5 gm. $CaCl_2$ (anhydrous) in water and dilute to 500 ml.
3. Silver nitrate—1% solution
   Dissolve 1 gm. $AgNO_3$ in water and dilute to 100 ml.

Procedure

Weigh 50 gm. of a blended portion into an 800-ml. beaker. Add about 400 ml. water and boil for 1 hour, replacing water lost by evaporation. Transfer contents of beaker to 500-ml. volumetric flask and make up to volume at 20°. Shake well and filter through No. 4 Whatman paper into 500-ml. Erlenmeyer flask.

After mixing the sample by rotating the Erlenmeyer flask, pipette duplicate 100-ml. aliquots into 800-ml. beakers. Add 300 ml. water and 10 ml. 1N NaOH from a pipette, stirring constantly, and let stand overnight.

Add 50 ml. of 1N acetic acid, with stirring, and allow to stand for 5 minutes. Add 25 ml. of 1N $CaCl_2$ solution, with stirring. Allow to stand for 1 hour. Heat to boiling and boil for 1 minute. Filter through 41H Whatman paper (15.0 cm.) that was previously washed with hot water, oven-dried for 2 hours at 100°, cooled in a desiccator and weighed in a covered weighing dish. Wash precipitate with almost boiling water until chloride-free (test with $AgNO_3$). Transfer filter paper to original weighing dish. Dry overnight at 100°, cool in desiccator and weigh.

Calculations

$$\% \text{ Ca pectate} = \frac{\text{wt. of Ca. pectate} \times 100}{\text{wt. of sample}}$$

References

Carré, M. H., and D. Haynes. The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin in apples. Biochem. J. 16: 60-69. 1922.

TOTAL AND REDUCING SUGARS

(Lane and Eynon Method)

Principle

Invert sugar reduces the copper in Fehling's solution to red, insoluble cuprous oxide. The volume of the unknown sugar solution required to completely reduce a measured volume of Fehling's solution is determined by titration, using methylene blue as indicator.

Reagents

1. Fehling's solution

   Prepare by mixing equal volumes of reagents 2 and 3 immediately before use.

2. Copper sulfate solution

   Dissolve 69.28 gm. $CuSO_4.5H_2O$ in water, dilute to 1,000 ml. and if necessary filter through No. 4 Whatman paper.

3. Alkaline tartrate solution

   Dissolve 346 gm. Rochelle salt (potassium sodium tartrate, $KNaC_4H_4O_6.4H_2O$) and 100 gm. NaOH in water and make up to 1,000 ml.

4. Methylene blue indicator

   Dissolve 1 gm. methylene blue in 100 ml. of water.

5. Neutral lead acetate—45% solution

   Dissolve 225 gm. neutral lead acetate, $Pb(C_2H_3O_2)_2.3H_2O$, in water and dilute to 500 ml.

6. Potassium oxalate—22% solution

   Dissolve 110 gm. potassium oxalate ($K_2C_2O_4.H_2O$) in water and dilute to 500 ml. An excess of lead acetate in the sugar solution will result in an error in the titration. Determine the exact amount of potassium oxalate solution necessary to precipitate the Pb^{++} from 2 ml. of the lead acetate solution as follows: Into each of six 50-ml. beakers containing 25 ml. water, pipette 2-ml. aliquots of the lead acetate solution. To the beakers add 1.6, 1.7, 1.8, 1.9, 2.0 and 2.1 ml. potassium oxalate solution, respectively. Filter each through a 41H Whatman paper and collect the filtrates in 50-ml. Erlenmeyer flasks. To each of the filtrates add a few drops of potassium oxalate solution. The correct amount of potassium oxalate required is the smallest amount which, when added to 2 ml. of lead acetate solution, gives a negative test for lead in the filtrate, i.e., no precipitate forms.

   The equivalent volumes should be marked on the bottles and employed when the solutions are used in sugar determinations.

Preparing the Standard Sugar Solution

Weigh 9.5000 gm. of pure sucrose. Transfer to a 400-ml. beaker, add 100 ml. water and 5 ml. conc. HCl. Let stand 3 days at 20-25° to allow inversion to take place. Transfer to a 1,000-ml. flask and make up to volume at 20°. The 1% solution thus obtained is stable for several months.

Neutralize the sugar solution for titration as follows:

Pipette 50 ml. of the standard invert solution into a 200-ml. volumetric flask and add about 100 ml. water. Using phenolphthalein as indicator, add 20% NaOH until solution turns pink. Acidify with 1N HCl, adding it dropwise, until one drop causes the pink color to disappear. Make up to volume with water. Titrate against 10 ml. Fehling's solution as described

under "Standard Method of Titration." The equivalent volume of neutralized sugar solution is 20.37. The titration should be within ± 0.05 ml., i.e., 20.32 to 20.42 ml.

Method of Titration

With solutions of unknown concentration, the incremental or trial method is first employed. When the correct dilutions are established, subsequent titrations are performed by the standard method.

The Incremental Method of Titration

Pipette 10 ml. of the mixed Fehling's solution (reagents 2 and 3) into a 250-ml. flask. Add from the burette (Figure 2) 15 ml. of the sugar solution or a larger volume if it is known to be insufficient to completely reduce the quantity of Fehling's solution used. Mix and heat to boiling on a hot plate covered with a clean asbestos-filled gauze. (Note: Wrap adhesive tape on neck of flask to make it easier to hold when hot.) Boil for 15 seconds. If the color remains blue, indicating that the Fehling's solution is not completely reduced, make further 3- to 5-ml. additions of the sugar solution. Boil the solution for a few seconds after each addition until it is judged unsafe (i.e., only the faintest perceptible blue color remains) to add more sugar solution without risk of passing the end point. Add 2 drops of methylene blue solution and complete the titration by adding the sugar solution dropwise until the indicator is completely decolorized. Record the volume of solution required. The accuracy of the Incremental Method is increased by approaching the end point as rapidly as possible and keeping as nearly as possible to a total boiling period of 3 minutes.

Standard Method of Titration

Pipette 10 ml. of mixed Fehling's solution (reagents 2 and 3) into duplicate 250-ml. Erlenmeyer flasks. Fill the 50-ml. burette with the solution to be titrated. Run into the flask almost the whole volume required to reduce the Fehling's solution, so that not less than 0.5 ml. or more than 1.0 ml. is required later to complete the titration. Mix the contents of the flask. Heat to boiling and boil moderately for 2 minutes, then add 2 drops of the methylene blue solution, taking care not to allow it to touch the side of the flask. Complete the titration within 1 minute by adding 2 to 3 drops of sugar solution at 5- to 10-second intervals, until the indicator is completely decolorized. At the end point the boiling liquid assumes the brick-red color of precipitated cuprous oxide, which it had before the indicator was added.

Procedure

Place 50 gm. of the blended jam in an 800-ml. beaker and add 400 ml. of water. To prevent inversion of sugars during boiling extraction, neutralize the solution to pH 7.5-8 with 0.1N NaOH, using a pH meter.

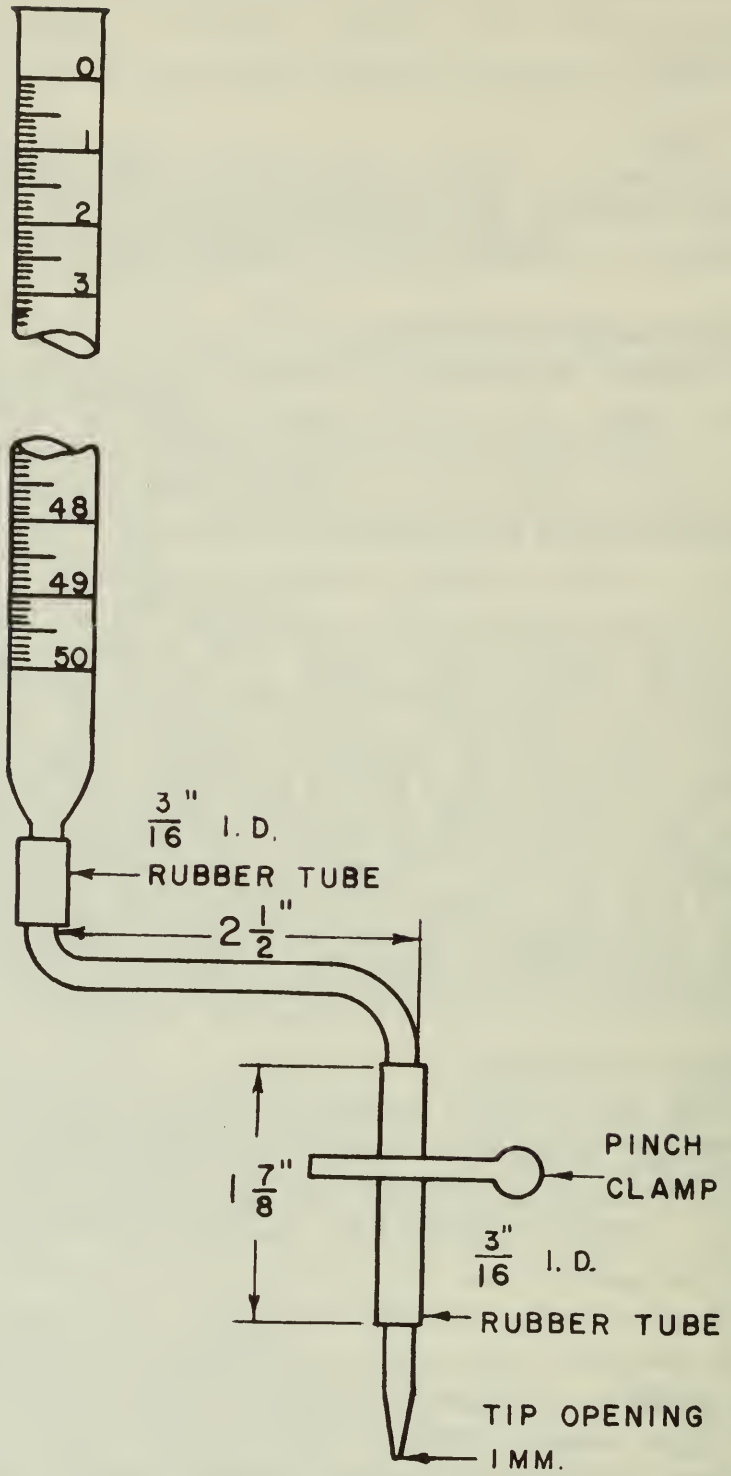

Figure 2.—Burette for sugar titration.

Boil gently for 1 hour, with occasional stirring. Add boiling water to maintain the original level. Cool, and transfer to a 500-ml. volumetric flask. Make up to volume and filter through No. 4 Whatman paper. Pipette a 100-ml. aliquot into a 500-ml. volumetric flask. Add 2 ml. of neutral lead acetate solution and about 200 ml. of water. Let stand for 10 minutes, then precipitate the excess Pb^{++} with potassium oxalate as follows: add the required amount of potassium oxalate solution as previously determined, make up to volume, shake well and filter through 41H Whatman paper. Test the filtrate for unprecipitated Pb^{++} with a drop of potassium oxalate. If precipitate forms, add 2 drops of potassium oxalate solution. Refilter and retest for Pb^{++}.

Reducing sugars.—Pipette 50 ml. of the clarified solution into a 100- or 250-ml. volumetric flask. (The dilution depends on the concentration of reducing sugars present.) Make up to volume and titrate by the "Standard Method."

Total sugars.—Pipette 50 ml. of the clarified solution into a 250-ml. Erlenmeyer flask. Add 5 gm. of citric acid and 50 ml. of water. Boil gently for 10 minutes to invert sucrose, then cool. Transfer to a 250-ml. volumetric flask and neutralize as under "Preparation of Standard Sugar Solution." Make up to volume and titrate by the "Standard Method."

Calculations

Reducing sugar

$$\% \text{ reducing sugar} = \frac{\text{factor}}{\text{titer}} \times \frac{100}{1000} \times \text{dilution}$$

Using factor from Table 3

If 24.6 ml. solution were required for titration:

$$\% \text{ reducing sugar} = \frac{51.2}{24.6} \times \frac{100}{1000} \times 250$$

$$= \frac{51.2}{24.6} \times 25$$

Total sugar

If 17.8 ml. solution were required for titration:

$$\% \text{ total sugar} = \frac{50.7}{17.8} \times 25$$

References

Atkinson, F.E., and C.C. Strachan. Candying of fruit in B.C. with special reference to cherries. Fruit Prod. J. 20: 132, 166, 199, 229, 262, 310. 1941.

Table 3. – Factors for 10 ml. of Fehling's Solution to be used with Lane and Eynon Volumetric Method[1]

Titer in ml.	Invert sugar no sucrose	Titer in ml.	Invert sugar no sucrose
15	50.5	33	51.7
16	50.6	34	51.7
17	50.7	35	51.8
18	50.8	36	51.8
19	50.8	37	51.9
20	50.9	38	51.9
21	51.0	39	52.0
22	51.0	40	52.0
23	51.1	41	52.1
24	51.2	42	52.1
25	51.2	43	52.2
26	51.3	44	52.2
27	51.4	45	52.3
28	51.4	46	52.3
29	51.5	47	52.4
30	51.5	48	52.4
31	51.6	49	52.5
32	51.6	50	52.5

[1] Association of Official Agricultural Chemists, Official methods of analysis. 8th ed. p. 906, Washington, D.C. 1955.

ASH

Principle

The dried sample is ignited at 525° to a white ash.

Procedure

Weigh duplicate 5- to 10-gm. blended samples into 100-ml. flat-bottom platinum or porcelain dishes. Heat on water bath or oven at 90° until water is expelled. Place slowly in muffle furnace at 525° and leave until white ash is obtained. Cool in desiccator and weigh. If black specks appear when water is added to the ash, the sample must be redried and placed in the furnace until a completely white ash is obtained. The time required varies with different products and must be determined by experiment.

TOTAL SOLIDS

Principle

A weighed portion of material is dried in a vacuum oven at a temperature not exceeding 70°. Drying time is determined by experiment and is considered sufficient when weighings made at 2-hour intervals do not differ by more than 3 mg.

Procedure

Weigh accurately into large (7 cm.) flat-bottom dishes duplicate 20-gm. blended samples, or a quantity that will give not more than 3 to 4 gm. of dry material. If necessary, to get a thin layer of the material, add a few ml. of water and mix thoroughly. Evaporate to dryness on a water bath and dry at 70° in a vacuum oven at 26 inches or higher vacuum until consecutive weighings made at intervals of 2 hours do not vary more than 3 mg. Overnight drying is usually sufficient for most samples.

Calculations

$$\% \text{ total solids} = \frac{\text{dry wt.}}{\text{wt. of sample}} \times 100$$

FRUIT JUICES

ASCORBIC ACID

(Indophenol Method)

Principle

Aliquots in oxalic acid solution are titrated with standardized sodium 2:6-dichlorophenolindophenol dye to a faint pink color that persists for 5 to 10 seconds. This method is limited to juices of light color because red pigments obscure the end point.

Reagents

1. Indophenol dye—0.04%

   Weigh 0.2 gm. sodium 2:6-dichlorophenolindophenol. Dissolve in about 200 ml. water, if necessary filter through No. 4 Whatman paper into 500-ml. volumetric flask and make up to volume at 20°. Store in refrigerator.

2. Oxalic acid—0.4%

   Dissolve 4 gm. oxalic acid in water and dilute to 1,000 ml.

Standardization of dye.—Dissolve 2 to 3 gm. potassium iodide in about 5 ml. water in 50-ml. Erlenmeyer flask (triplicate). Add 15 ml. dye with a pipette and then 10 ml. 1N HCl. Mix and let stand for 2 minutes. Titrate with freshly prepared 0.01N sodium thiosulfate from a microburette (20 ml. 0.1N in 200-ml. volumetric flask at 20°), using 1 to 2 ml. starch, until there is no change in color when one drop or less is added. Complete the titration in 1 minute. The dye should be standardized every 48 hours and kept not more than two weeks. Store the dye solution in a refrigerator.

Calculations

If 15 ml. dye required 3.21 ml. sodium thiosulfate:

$$1 \text{ ml. dye} = \frac{1}{1000} \times \frac{\text{ml. } Na_2S_2O_3 \times \text{normality of } Na_2S_2O_3 \times 88 \times 1000}{\text{ml. dye}}$$

$$= \frac{1}{1000} \times \frac{3.21 \times 0.01 \times 88 \times 1000}{15}$$

$$= 0.188 \text{ mg. ascorbic acid}$$

Procedure

Shake can well, determine vacuum using a gauge. Pipette 10 ml. juice (25 ml. for juices low in vitamin C) into 100-ml. volumetric flask, make up to volume with 0.4% oxalic acid and filter through No. 4 Whatman filter paper (clarified juices need not be filtered). Pipette a 5- or 10-ml. aliquot for titration, depending on amount of ascorbic acid present. Add about 15 ml. oxalic acid (0.4%) and titrate in a 50-ml. Erlenmeyer flask with 0.04%

dye to a faint pink end point lasting for *5 to 10 seconds*. Titration must be completed within 1 minute and the total dye required should not exceed 1.5 ml. A microburette should be used for the dye.

Calculations

mg. ascorbic acid per 100 ml. juice = dye equivalent × titer × dilution.
If 1.05 ml. dye were required for titration:
mg. ascorbic acid per 100 ml. juice = 0.188 × 1.05 × 200
= 39.4

Rapid Method for Tomatoes

Blend about 500 gm. of tomatoes in a blendor for not more than 5-7 seconds. Weigh a 50-gm. sample and using 0.4% oxalic acid transfer to a 250-ml. volumetric flask. Make up to volume with 0.4% oxalic acid and filter through No. 4 Whatman paper. Using a 5- or 10-ml. aliquot of filtrate, titrate as above with indophenol dye.

References

Bessey, O.A., and C. G. King. The distribution of vitamin C in plant and animal tissues, and its determination. J. Biol. Chem. 103: 687-698. 1933.

Strachan, C. C. Factors influencing ascorbic acid retention in apple juice. Canada Dept. Agr. Pub. 732. Tech. Bull. 40. 1942.

ASCORBIC ACID

(Colorimetric Method)

Principle

Ascorbic acid is extracted from the material in a Waring blendor using oxalic acid. The decolorizing effect of the extracted ascorbic acid on indophenol dye is measured with a photoelectric colorimeter.

Apparatus

1. Photoelectric colorimeter
   With a Klett-Summerson, use filter No. 540.
2. Matched tubes
   If matched tubes are not available, mark 4 tubes as follows:
   DW – distilled water
   S – standard (also used later for unknown solution)
   No. 1
   No. 2

Use these tubes in the required sequence throughout the procedure.

Reagents

1. Stock ascorbic acid solution—0.1%

   Dry ascorbic acid crystals over sulfuric acid. Dissolve 0.2 gm. in 0.4% oxalic acid and dilute to 200 ml. with 0.4% oxalic acid.

2. Working standards (W.S.)

   Take 5, 10, 15, 20 and 25 ml. of stock ascorbic acid solution and make each up to 500 ml. with 0.4% oxalic acid. Resulting solutions numbered 1 to 5 contain 1, 2, 3, 4 and 5 mg. ascorbic acid per 100 ml. respectively. Check each solution against standard iodine or 0.04% indophenol dye (use 50 ml. W.S. with 0.01N iodine and 20 ml. W.S. with dye).

3. Dye—sodium 2:6-dichlorophenolindophenol

   Dilute 30 ml. 0.04% dye to 1,000 ml. (12.0 mg./liter) (0.0012%).

Standardization

To the four matched tubes add reagents as follows:

DW – 10 ml. distilled water
S – 1 ml. W.S. No. 1 + 9 ml. water. Mix.
No. 1 – 1 ml. 0.4% oxalic acid
No. 2 – 1 ml. W.S. No. 1

Adjust galvanometer to zero with tube DW in instrument. To tube No. 1, add 9 ml. dye, invert to mix, insert into instrument in place of DW and take galvanometer reading in 15 seconds. Record as L_1.

Adjust galvanometer to zero with tube S in instrument. To tube No. 2 add 9 ml. dye, invert to mix, insert into instrument in place of tube S and take galvanometer reading in 15 seconds. Record as L_2.

In succession, record L_1 and L_2 readings for each working standard, rinsing the tubes with distilled water and drying between each determination.

On graph paper, against concentration of ascorbic acid in mg. per 100 ml. as abscissa, plot readings $L_1 - L_2$ for each working standard. Draw standard curve.

Preparing the Sample

Weigh 350 gm. 0.4% oxalic acid into blendor jar. Add exactly 50 gm. of representative portion of solid material. Blend for 3 minutes and filter through No. 4 Whatman paper. If material is juice, pipette 50 ml. into 250-ml. volumetric flask and make up to volume, using 0.4% oxalic acid. Filter if necessary and mark as filtrate.

Procedure

Obtain reading L_1 as described under "Standardization."

To tube S add 1 ml. filtrate + 9 ml. water, mix and adjust galvanometer to zero.

To tube No. 2 add 1 ml. filtrate + 9 ml. dye, invert to mix, insert into instrument in place of tube S and take reading in 15 seconds. Record as L_2.

From the standard curve find the concentration of ascorbic acid in mg. per 100 ml. filtrate corresponding to $L_1 - L_2$.

Calculations

mg. ascorbic acid per 100 gm. = ascorbic acid in mg. per 100 ml. filtrate × dilution.

Note: Unknown must contain 0.01 to 0.05 mg. ascorbic acid per ml.

References

Bessey, O.A. A method for the determination of small quantities of ascorbic acid and dehydroascorbic in turbid and colored solutions in the presence of other reducing substances. J. Biol. Chem. 126: 771-784. 1938.

Evelyn, K. A., H. T. Mallory and C. Rosen. The determination of ascorbic acid in urine with the photoelectric colorimeter. J. Biol. Chem. 126: 645-654. 1938.

Loeffler, H.J., and J. D. Ponting. Ascorbic acid. Rapid determination in fresh, frozen or dehydrated fruits and vegetables. Ind. Eng. Chem. Anal. ed. 14: 846-849. 1942.

TOTAL AND REDUCING SUGARS

(Lane and Eynon Method)

Reagents—See "Jams, Jellies and Marmalades."

Procedure

Weigh 25 gm. filtered (Whatman No. 4) sample and transfer to 400-ml. beaker. Add about 100 ml. water, neutralize to pH 7.5-8 with 1N NaOH and transfer into 250-ml. volumetric flask. Add 100-200 ml. water and 2 ml. lead acetate solution. Shake and let stand for 10 minutes. Add the necessary amount of potassium oxalate, bring up to volume with water and filter through 41H or No. 5 Whatman paper. Test filtrate with small amount of potassium oxalate to determine if lead is absent. Mark filtrate "A."

Total sugar

Pipette 50 ml. filtrate A into a 250-ml. Erlenmeyer flask. Add 5 gm. citric acid and 50 ml. water. Boil gently for 10 minutes to invert the sucrose, then cool. Transfer to a 250-ml. volumetric flask or a 250-ml. beaker and neutralize, using phenolphthalein as indicator. Add 20% NaOH until solution turns pink. Add 1N HCl dropwise until the pink color disappears. If red color obscures the end point, adjust on pH meter to 8.1. Make up to volume in a 250-ml. flask and titrate by the "Standard Method" under "Jams, Jellies and Marmalades."

Calculations

$$\% \text{ total sugar} = \frac{\text{factor}}{\text{titer}} \times \text{dilution} \times \frac{100}{1000}$$

$$= \frac{\text{factor (from Table 3)}}{\text{titer}} \times 5.0$$

Reducing sugar

For most juices, reducing sugars are low and therefore filtrate A can be used directly in the "Standard Method" of titration as directed under "Jams, Jellies and Marmalades."

Calculations

$$\% \text{ reducing sugar} = \frac{\text{factor}}{\text{titer}} \times \text{dilution} \times \frac{100}{1000}$$

$$= \frac{\text{factor (from Table 3)}}{\text{titer}} \times 1.0$$

TOTAL ACIDITY

Principle

The total acidity is determined by titrating a diluted sample of juice with standard NaOH to pH 8.1 using a pH meter.

Procedure

If juice has not been clarified previously, filter through No. 4 Whatman paper. Pipette 10 ml. of juice into 250-ml. beaker. Add about 100 ml. water and titrate with 0.1N NaOH to pH 8.1, using a pH meter.

Calculation

Calculate percent total acidity as the predominant acid present as outlined under acidity of "Jams, Jellies and Marmalades."

SPECIFIC GRAVITY

Principle

Specific gravity is determined on a sample of juice, using a hydrometer at the temperature specified.

Procedure

Cool about 200 ml. juice in 250-ml. Erlenmeyer flask to exact temperature specific for the hydrometer. Rinse hydrometer graduate with about 50 ml. of cooled juice. Fill graduate with juice, insert the hydrometer and take reading. Read the hydrometer at the liquid surface level, not at the top of meniscus. Make sure hydrometer is floating freely when read.

SOLUBLE SOLIDS

Procedure

Take soluble solids reading at 20°, using a refractometer. For apple juice, the percent soluble solids multiplied by 4 should be about equal to the last two figures of the specific gravity reading.

Example: Soluble solids = 12.0%
Specific gravity should be close to 1.048

pH

Principle

The pH as measured directly with a pH meter indicates the effective acidity of the juice.

Procedure

Standardize the pH meter with pH 4.0 buffer.

Pour juice in 50-ml. beaker and determine pH. This sample can be used for evaluating flavor, color, aroma and clarity.

CANDIED FRUIT AND PEEL

PREPARING THE MATERIAL

Pass material for analysis through a food chopper and mix thoroughly with a spoon. Products with a liquid portion can be blended. Place in an airtight container and store until ready for analysis.

TOTAL AND REDUCING SUGARS

(Lane and Eynon Method)

Procedure

Proceed as under "Total and Reducing Sugars" in "Jams, Jellies and Marmalades."

TOTAL ACIDITY

Procedure

Using a 50-gm. sample, proceed as under "Jams, Jellies and Marmalades." Calculate acidity as citric acid.

SODIUM BENZOATE

Procedure

Proceed as under "Mincemeat."

SULFUR DIOXIDE

Procedure

Using a 50-gm. sample, proceed as under "Jams, Jellies and Marmalades."

ARTIFICIAL FOOD DYES

Procedure

Proceed as under "Jams, Jellies and Marmalades."

SOLUBLE SOLIDS

Procedure

If the product has free syrup, use a portion of the syrup and take a soluble solids reading with a refractometer. Temperature correction (Table 1) should be applied if the instrument is not at 20°.

DEHYDRATED FRUITS AND VEGETABLES

Dehydrated Apples

PREPARING THE MATERIAL

Pass the material for analysis through a food chopper and mix thoroughly, completing operation as quickly as possible to avoid absorption of moisture or loss of sulfur dioxide. Replace material in airtight container and hold until ready for analysis.

MOISTURE

Procedure

Spread about 10 gm. of prepared sample over the bottom of a weighed aluminum dish provided with a tightly fitted cover (7-cm. diam.). Begin weighing with the cover off while adding the sample, until slightly over 10 gm. Close the dish and weigh accurately and as rapidly as possible.

Dry for 6 hours at 70° and 26-28 inches vacuum. Remove the dish cover during this operation. During the drying, admit to oven a slow current of air (2 bubbles per second) dried by passing through sulfuric acid. Replace the cover, cool dish in desiccator and weigh.

Calculations

$$\% \text{ moisture} = \frac{\text{loss in wt.}}{\text{wt. of sample}} \times 100$$

SULFUR DIOXIDE

Procedure

Using a 25-gm. sample, proceed as outlined for "Jams, Jellies and Marmalades." Boiling time is 1 hour.

Dehydrated Potatoes, Carrots, Etc.

MOISTURE

Preparing the Material

Comminute material for analysis in Waring blendor for 1 to 2 minutes. Avoid overheating it. Pass through No. 60 mesh sieve and place in an airtight container.

Procedure

Weigh duplicate 2- to 3-gm. samples into previously dried and weighed aluminum dishes (7-cm. diam.). Place in vacuum oven and dry under the same conditions as outlined for "Dehydrated Apples."

Calculations

$$\% \text{ moisture} = \frac{\text{loss in wt.}}{\text{wt. of sample}} \times 100$$

MINCEMEAT

SODIUM BENZOATE

Principle

In a sodium chloride solution containing an excess of Na^+, by addition of sodium hydroxide, benzoic acid is converted into water-soluble sodium benzoate. When the sodium benzoate solution is acidified with excess hydrochloric acid, water-insoluble benzoic acid is formed. The benzoic acid is extracted with chloroform. The chloroform is removed by evaporation and the residue containing benzoic acid is dissolved in alcohol and then titrated with standard sodium hydroxide.

Preparing the Material

Prepare dry materials such as candied fruit by passing them through a food chopper; pickles, mincemeat, etc., can be blended. Place materials in airtight containers and keep for analysis.

Procedure

Weigh a 100-gm. sample and transfer to 500-ml. volumetric flask with water (use a powder funnel). Add 10 ml. NaOH (10%) and enough sodium chloride to saturate the solution (30 gm. NaCl for every 100 ml. solution). Adjust volume of liquid to about 400 ml. and allow to stand 2 hours, with frequent shaking.

Make up to volume with water and filter through No. 4 Whatman paper into a 600-ml. beaker. Pipette 100 ml. filtered extract into 500-ml. shaking bottles and neutralize with HCl (1 + 3). If 10 ml. NaOH was added previously, the amount of HCl required is 2 ml. Add 5 ml. excess or a total of 7 ml. HCl (1 + 3). Add 50 ml. chloroform to each bottle, close cap and shake gently, releasing the cap periodically to release any pressure. Shake and let stand alternately for 30 minutes. Shaking should be sufficient for proper mixing but not violent enough to cause an emulsion. This point varies with the product.

After 30 minutes, transfer solution slowly to a 500-ml. separatory funnel. When layers have separated, draw off 25 ml. of the lower layer and transfer to a 250-ml. beaker. Allow to stand at room temperature until chloroform has evaporated. Dissolve residue by adding 50 ml. alcohol (4 + 1). Add 50 ml. water and titrate on a pH meter to pH 8.1, using 0.05N sodium hydroxide from a 5-ml. microburette. Run a blank titration on a solution of alcohol (4 + 1) and adjust results accordingly.

Calculations

1 ml. 0.05N NaOH = 0.0072 gm. anhydrous sodium benzoate

If 1.2 ml. NaOH were required for titration:

$$\text{p.p.m.} = \frac{\text{titer} \times \text{normality} \times 0.0072 \times \text{dilution factor} \times 1{,}000{,}000}{0.05 \times \text{sample wt.}}$$

$$= \frac{1.2 \times 0.05 \times 0.0072 \times 10 \times 1{,}000{,}000}{0.05 \times 100}$$

$$= 1.2 \times 0.05 \times 14{,}400$$

PICKLES

SODIUM BENZOATE

Procedure

See section under "Mincemeat."

SODIUM CHLORIDE

(Chromate Indicator Method)

Principle

An aliquot taken from a neutralized solution containing sodium chloride is titrated with a standardized solution of silver nitrate using potassium chromate as an indicator.

Reagents

1. Silver nitrate– 0.1N

   Dissolve 4.3 gm. reagent-grade silver nitrate in water and dilute to 250 ml. in a volumetric flask. Standardize against a solution containing 0.50 gm. sodium chloride (dried at 110° before weighing) per 100 ml. water.

2. Potassium chromate

   Dissolve 5 gm. potassium chromate in water and dilute to 100 ml.

Procedure

Take either a 20-gm. or 20-ml. sample. Add about 100 ml. water and neutralize to pH 5 to 7 with dilute sodium hydroxide. If a pH meter is not available, add methyl orange and enough alkali to change indicator from orange to yellow. Transfer the solution to 200-ml. volumetric flask, make up to volume, mix and filter through No. 4 Whatman paper. Pipette 50-ml. aliquot of filtrate into 150-ml. Erlenmeyer flask, add 1 ml. of potassium chromate solution and titrate with standard silver nitrate solution. The end point is the first permanent red color.

Good lighting should be provided for the titration since the end point is extremely hard to detect with poor illumination. The analyst should satisfy himself that he is able to reproduce his results with a satisfactorily high degree of precision, since certain individuals are color blind to the particular color changes involved.

Calculations

$$\% \text{ NaCl} = \frac{\text{ml. } AgNO_3 \times \text{N } AgNO_3 \times \text{equiv. wt. of NaCl} \times 100}{1000 \times \text{wt. of sample}}$$

$$= \frac{\text{ml. } AgNO_3 \times \text{N } AgNO_3 \times 58.45 \times 100}{1000 \times 5 \text{ (where dilution is 4)}}$$

If the sample is taken by volume rather than by weight, report as percent w/v.

References

National Canners Association. Laboratory manual for the canning industry. Section 21, p. 13. National Canners Association, Washington, D. C. 1954.

WINES AND CIDER

PREPARING THE MATERIAL

Before taking a sample of carbonated drinks for subsequent procedures it is good practice to pour the sample from one beaker to another numerous times. This helps to expel CO_2 and removes excess bubbles. If an abnormal amount of acetic acid is present, neutralize with 1N NaOH.

ALCOHOL

(Pycnometer Method)

Apparatus

1. Constant-temperature water bath.
2. Pycnometers, 50 ml. and 100 ml.
3. Distillation apparatus with 500-ml. flask and 40-cm. condenser.

Calibration

Clean pycnometer and fill with distilled water. Place in water bath at temperature marked on pycnometer (usually 60° F.) and leave for 15 minutes. Adjust water level in flask until bottom of meniscus is exactly on graduation mark. With a piece of filter paper dry inside neck of pycnometer. Place back into bath for 10 minutes. Remove flask from water bath, dry, let stand for 10 minutes at room temperature and weigh. Empty pycnometer, rinse with acetone and dry with air stream. Let flask come to room temperature, stopper and weigh.

Procedure

Fill a dry 100-ml. pycnometer with sample and adjust to temperature specified. Transfer contents to the distillation flask. Rinse pycnometer three times, using a total of 50 ml. cold water, adding the rinsing to the flask. If foaming is expected, add a small amount of tannin. Complete distillation connections and distill into pycnometer flask until a volume of about 95-98 ml. has been collected. Remove pycnometer and place in water bath at constant temperature specified on pycnometer. After 15 minutes dilute exactly to the mark, using water at the same temperature. Dry inside neck and outside of pycnometer and then weigh.

Calculations

$$\text{sp.gr. of distillate} = \frac{\text{wt. pycnometer with distillate} - \text{wt. empty pycnometer}}{\text{wt. pycnometer with water} - \text{wt. empty pycnometer}}$$

From an alcohol table for 60° F. find the corresponding alcohol content (% by volume).

ALCOHOL

(By Distillation and Hydrometer)

Principle

A measured volume of sample is distilled and the distillate diluted to a definite volume, usually the original volume. The alcohol content of the distillate is determined by means of a hydrometer.

Procedure

Pipette 100-ml. sample into the distillation flask and add 50 ml. water. Add a small amount of tannin if foaming occurs. (Note: A small amount of added Antifoam "A" (Dow Corning) prevents foaming.) If an abnormal quantity of acetic acid is present, neutralize exactly with 1N NaOH. Place a 250-ml. Erlenmeyer flask in position to collect the distillate. With low heat distill about 90 to 95 ml. into the flask. Remove the flask, transfer distillate to a 200-ml. volumetric flask, cool to required temperature and make up to volume with water.

Note the temperature on the stem of the hydrometer. Cool the distillate to *exactly* this temperature in an ice bath. Fill the hydrometer cylinder with distillate, check temperature and insert hydrometer. Read the percent alcohol at the bottom of the meniscus, that is, at the general level of the liquid. Multiply the result by the dilution factor to obtain the percentage of alcohol.

A specific gravity hydrometer may be used instead of one reading in percent alcohol. Tables are available to convert specific gravity of alcohol – water mixtures to percent alcohol by volume.

TOTAL ACIDITY

Principle

Total acidity is determined by direct titration with 0.1N NaOH to pH 8.1, using a pH meter.

Procedure

Weigh 10-gm. sample into a 250-ml. beaker. Add 100 ml. water and bring quickly to boil to expel CO_2. Do not continue boiling because volatile acids may be lost. Cool the sample and titrate to pH 8.1 with 0.1N NaOH, using a pH meter. Record volume of NaOH required and calculate the total acidity as percent of the predominant acid.

Calculations

1 ml. 0.1N NaOH = 0.0075 gm. tartaric acid

If 5.8 ml. 0.1N NaOH were required for titration:

$$\% \text{ total acid} = \frac{1}{10} \times \frac{\text{equiv. wt. of acid} \times \text{normality of NaOH} \times \text{titer}}{\text{wt. of sample}}$$

$$= \frac{1}{10} \times \frac{75.0 \times 0.1 \times 5.8}{10}$$

Note: See page 3 for equivalent weights of acids.

TANNIN AND COLORING MATTER

Principle

A de-alcoholized sample is titrated with standard potassium permanganate using indigo solution as an indicator. The volume of permanganate solution required minus that required to oxidize a similar aliquot from which the tannin and coloring matter were removed is the volume of permanganate required to oxidize the tannin.

Reagents

1. Potassium permanganate–0.1N

   Dissolve 3.160 gm. in about 200 ml. water. Transfer to 1,000-ml. volumetric flask and make up to volume with water at 20°. Standardize with sodium oxalate as on page 46.

2. Indigo solution

   Dissolve 3 gm. sodium indigotin disulfonate in 200 ml. water by heating, cool, add slowly 25 ml. conc. H_2SO_4. Transfer to 500-ml. volumetric flask and dilute to mark with water.

3. Purified boneblack

   Boil 200 gm. powdered boneblack with two successive portions of HCl (1 + 3). Filter through hard filter paper, wash with boiling water until chloride-free (test with $AgNO_3$). Place in flask and keep covered with water. If acid-washed charcoal is available, this step can be eliminated.

Procedure

Pipette 100 ml. wine or cider into 400-ml. beaker. Add about 25 ml. water and remove alcohol by evaporating on a hot plate to about 75 ml. Cool, transfer to 100-ml. volumetric flask, rinsing the beaker several times. Cool and make up to volume.

Pipette 10 ml. of the de-alcoholized sample into a 2,000-ml. porcelain evaporating dish. Add 1,000 ml. water and exactly 20 ml. indigo solution (reagent 2). Add the standard $KMnO_4$ solution from a burette 1 ml. at a time until the blue color changes to green, then add a few drops at a time until the color becomes golden yellow. Designate number of ml. of $KMnO_4$ solution as "a."

Transfer remaining de-alcoholized sample from the volumetric flask to a 250-ml. Erlenmeyer flask. Add about 1 gm. prepared boneblack and after 10 minutes, with occasional shaking, filter through No. 41H or No. 5 paper. The carbon adsorbs tannin, other nontannins and anthocyanins. The filtrate must be crystal clear.

Pipette 10 ml. of decolorized sample into a 2,000-ml. porcelain evaporating dish. Add 1,000 ml. water and exactly 20 ml. indigo solution. Titrate with the $KMnO_4$ solution as directed above. Designate ml. $KMnO_4$ as "b."

Calculations

1 ml. 0.1N $KMnO_4$ = 0.00416 gm. tannin and coloring matter.

$$\% \text{ tannin and coloring matter} = \frac{(a - b) \times \text{normality of } KMnO_4 \times 4.16}{\text{wt. of sample titrated}}$$

References

Cruess, W. V., M. A. Joslyn and L. G. Saywell. Laboratory examination of wines and other fermented products, pp. 66-69. Avi Publishing Co. Inc., New York, N.Y. 1934.

TOTAL AND REDUCING SUGARS

(Total Sugar – Dry Wines and Cider)

Reagents

See "Jams, Jellies and Marmalades."

Preparing the Sample

Weigh 200-gm. sample into 400-ml. beaker, neutralize with 1N NaOH to pH 8.1 on meter and evaporate to about 100 ml. Cool, transfer to 200-ml. volumetric flask and make up to volume at 20°. Transfer to 400-ml. beaker, add about 1 gm. decolorizing charcoal, shake, and let stand for 10 minutes. If necessary add one teaspoon of Hyflo Super-cel or similar filter aid, shake and filter through 41H paper. The filtrate must be clear and if so may be used directly in the "Standard Method" titration as under "Jams, Jellies and Marmalades." This applies where it can be safely assumed that the sugar present is all reducing sugar.

Calculations

$$\% \text{ sugar} = \frac{\text{factor (from Table 3)}}{\text{titer}} \times \frac{100}{1000}$$

(Total and Reducing Sugar – Sweet Wines and Cider)

Preparing the Sample

Weigh 25-gm. sample into 400-ml. beaker. Add about 150 ml. water and neutralize to pH 7.5-8.0 with 1N NaOH. Evaporate to about 100 ml. (20-25 minutes' boiling). Cool, transfer to 250-ml. volumetric flask, add 100-200 ml. water and 2 ml. lead acetate solution. Shake, and let stand for 10 minutes. Add the necessary amount of potassium oxalate (see "Jams, Jellies and Marmalades"), bring up to volume with water and filter through 41H or No. 5 paper. Test filtrate with small amount of potassium oxalate to determine if lead is absent. If filtrate is clear, follow the procedure for "Total and Reducing Sugars," under "Fruit Juices," using 50-ml. aliquots.

Calculations

See "Total and Reducing Sugars," under "Fruit Juices."

References

Cruess, W.V., M.A. Joslyn and L. G. Saywell. Laboratory examination of wines and other fermented products. pp. 52-58. Avi Publishing Co. Inc., New York, N.Y. 1934.

IRON

(Colorimetric Method)

Wet Ashing

Reagents

1. Nitric acid – concentrated
2. Perchloric acid – 72% $HClO_4$

Procedure

Pipette 25 ml. of thoroughly degassed sample into 125-ml. Erlenmeyer flask and boil down to a thick syrup, slightly charred. Add 20-25 ml. HNO_3 and 2 ml. $HClO_4$ to flask and heat gently in fume cabinet until initial reaction begins. This is indicated by vigorous boiling with evolution of brown nitrogen tetroxide.

After reaction has subsided, again heat contents of the flask to slow boiling and continue boiling until all HNO_3 is driven off as evidenced by evolution of fumes of $HClO_4$. When cooled, the residue should be colorless or at the most a pale yellow. If not, add small amounts of HNO_3 and $HClO_4$ and heat further. After contents are completely ashed and cooled, transfer to 100-ml. volumetric flask and make up to volume.

Preparation of Standard Curve

Reagents

1. Glass-distilled water

2. Acetic acid–2M

Dilute 120 gm. acetic acid to 1 liter with water.

3. Ammonium citrate solution–1%

Dissolve 1 gm. ammonium citrate in water and dilute to 100 ml.

4. Bromophenol blue indicator–0.04%

Dissolve 0.1 gm. bromophenol blue in 3 ml. 0.05N NaOH, transfer to a volumetric flask and dilute to 250 ml. with water.

5. Buffer solutions

(a) pH 3.5 – Mix 6.4 ml. 2M sodium acetate with 93.6 ml. 2M acetic acid and dilute to 1 liter.

(b) pH 4.5 – Mix 43 ml. 2M sodium acetate with 57 ml. 2M acetic acid and dilute to 1 liter.

6. Hydroquinone solution–1%

Dissolve 1 gm. hydroquinone in 100 ml.buffer solution (pH 4.5). Store in refrigerator and discard as soon as any color develops.

7. *o*-Phenanthroline solution

Dissolve 1 gm. *o*-phenanthroline monohydrate in water and dilute to 400 ml.

8. Sodium acetate solution–2M

Dissolve 272 gm. $NaC_2H_3O_2.3H_2O$ in water dilute to 1 liter.

9. Standard iron solution – No. 1 – 1 mg. Fe/ml.

Dissolve 1 gm. electrolytic iron in 50 ml. 10% H_2SO_4. Cool and dilute to 1 liter with water.

10. Standard iron solution–No. 2

Pipette 5 ml. of standard iron solution No. 1 into 500-ml. volumetric flask and make up to volume with water. One ml. of this solution contains 0.01 mg. Fe. (10 mcg.)

Pipette 2-, 4-, 6-, 8- and 10-ml. aliquots standard iron solution No. 2 into 25-ml. volumetric flasks and the same amounts into test tubes. Add 5 drops bromophenol blue indicator to aliquots in the test tubes and add sodium acetate solution until color matches that of equal volume of buffer solution of pH 3.5 containing same quantity of indicator. Add 1 ml. of hydroquinone solution and 2 ml. *o*-phenanthroline solution to the aliquots in the volumetric flasks. Adjust pH of the contents to 3.5 by adding same volume of sodium acetate solution as found necessary for aliquot in test tubes. Prepare a blank according to above procedure but omitting standard iron solution No. 2. Make all 25-ml. volumetric flasks up to volume with water, mix thoroughly and plot optical density against concentration on graph paper at wave length of 510 mμ.

Procedure

Pipette 10 ml. of digested sample into 25-ml. volumetric flask and same amount into a test tube. Add 5 drops bromophenol indicator to aliquot in

test tube and titrate with sodium acetate solution until color matches that of equal volume of buffer solution of pH 3.5 containing the same quantity of indicator.

Add 1 ml. hydroquinone solution and 2 ml. *o*-phenanthroline solution to aliquot in volumetric flask. Adjust pH of contents to 3.5 by adding same volume of sodium acetate solution as was found necessary for aliquots in test tube. If turbidity develops upon adjustment of pH of aliquot in test tube, add 1 ml. NH_4 citrate solution to volumetric flask before adding the sodium acetate solution.

Prepare blank according to the above procedure. Make all flasks up to volume with water and mix thoroughly. Let stand for 1 hour to assure complete color development. Take colorimeter readings and plot optical density on the same graph paper as the standard curve.

Calculations

p.p.m. Fe = mcg. Fe from curve divided by 2.5.

References

Association of Official Agricultural Chemists. Official methods of analysis. 8th ed. Washington, D.C. 1955.

TOTAL VOLATILE ACIDITY

Principle

Volatile acids are steam-distilled from the sample using a Hortvet type distillation apparatus, and titrated with standard sodium hydroxide. The acids are calculated as acetic acid.

Procedure

Boil about 200 ml. of water in the outer heating flask. Most flasks are provided with an outside vent tube. The water may be boiled in the outer flask with the vent tube open to remove the dissolved CO_2, as well as to replace with steam the air in the flask surrounding the inner tube. Apply heat gently and turn on cold water through condenser.

With a pipette, introduce a 20-ml. sample into inner tube and connect at once to the condenser. Increase the heat and bring the water in flask to vigorous boiling, having the pinch cock on side of the tube open. When the water is boiling vigorously, close the pinch cock. Steam passes through the 20-ml. sample, carrying the volatile acid into the condenser. Collect the condensate in a 250-ml. Erlenmeyer flask. Continue distillation until 100 ml. is collected.

Transfer the 100 ml. of distillate to 250-ml. beaker. Add about 50 to 100 ml. of water and titrate with 0.1N NaOH to pH 8.1.

Calculations

1 ml. 0.1N NaOH = 0.006 gm. acetic acid

Note:

To determine whether all the volatile acids have been extracted, 50 ml. of distillate is first collected in one flask and titrated. An additional 10-ml. portion is then collected in a second flask and added to the distillate in the first flask and titrated. Further 10-ml. portions are distilled over until an additional 10-ml. portion does not change the titration by more than one or two drops. Generally only 80 ml. of distillate is required.

References

Cruess, W.V., M. A. Joslyn and L. G. Saywell. Laboratory examination of wines and other fermented products. pp. 33-44. Avi Publishing Co. Inc., New York, N.Y. 1934.

VOLATILE ACIDITY

(Exclusive of SO_2)

Principle

After removal of the free sulfur dioxide by addition of barium hydroxide, the volatile acids are steam-distilled from the sample and titrated with standard sodium hydroxide.

Procedure

Pipette 50-ml. sample into 250-ml. beaker, and add enough clear saturated $Ba(OH)_2$ solution to bring mixture to pH 8.1. Allow to stand 30 minutes and maintain at pH 8.1 by adding more $Ba(OH)_2$ if necessary. Transfer to 100-ml. volumetric flask, dilute to volume and filter immediately through No. 2 Whatman paper. Pipette 20 ml. of filtrate into inner tube of volatile acidity distillation flask and add 1 ml. of H_2SO_4 (1 + 3). Place 150 ml. recently boiled hot water in outer flask and distill 100 ml. Using pH meter, titrate with 0.1N NaOH to pH 8.1.

1 ml. 0.1N NaOH = 0.006 gm. acetic acid

References

Association of Official Agricultural Chemists. Official methods of analysis. 8th ed. p. 189. Washington, D.C. 1955.

EXTRACT

The "extract" of wine and cider represents the alcohol-free soluble solids present and consists mainly of tartaric acid, potassium bitartrate, malic acid, protein, coloring matter, sugar and gums.

One method of determining the extract is to de-alcoholize the sample by boiling, dilute to the original volume and determine specific gravity with a Brix or Balling hydrometer. The other method is to evaporate a measured volume of sample to dryness and weigh the extract.

(Hydrometer Method)

Procedure

Pipette 100-ml. sample into 400-ml. beaker. Add 50 ml. water and evaporate slowly to a volume of about 50 ml. Avoid loss by spattering. Transfer to 100-ml. volumetric flask and rinse the beaker with water. Add the washings to the flask. Cool and dilute to mark. Transfer to a cylinder and insert a Brix or Balling hydrometer. The reading gives the grams of extract per 100 ml. of original sample. (For approximate purposes the refractometer reading gives a satisfactory result.)

(Oven Method)

Procedure

Place empty evaporating dishes in oven at 100° for 1 hour. Transfer to desiccator, cool and weigh. Pipette 50-ml. sample into evaporating dish and heat on water bath until the liquid has evaporated to a viscous consistency. Place dishes in vacuum oven at 70° and 26 to 28 inches vacuum for 8 hours. Place in desiccator, cool and weigh.

Calculations

$$\% \text{ extract} = \frac{\text{wt. of extract} \times 100}{\text{wt. of sample}}$$

SAUERKRAUT

TOTAL ACIDITY

Procedure

From the liquid portion of the product weigh 5 gm. into 250-ml. beaker. Add 100 ml. water, boil for a few minutes to drive off CO_2, cool and titrate with pH meter to pH 8.1, using 0.1N NaOH.

Calculations

$$\% \text{ lactic acid} = \frac{\text{titer} \times N \times \text{equiv. wt.} \times 100}{1{,}000 \times \text{wt. of sample}}$$

If 7.2 ml. 0.1N NaOH were required for titration:

$$\% \text{ lactic acid} = \frac{7.2 \times 0.1 \times 90.08 \times 100}{1{,}000 \times 5}$$

$$= 1.3$$

pH

Procedure

Adjust pH meter with pH 4.0 buffer solution.

Pour 50 to 75 ml. of juice into 100-ml. beaker and take pH reading.

FRUIT PRESERVED IN SULFUR DIOXIDE

SULFUR DIOXIDE

(Official Method)

Preparing the Material

Place portion of material for analysis free from pits into Waring blendor jar. Blend just long enough to mix into a slurry. Place portion into an airtight container until ready for analysis.

Procedure

Using 25 gm. blended sample, follow the procedure for "Jams, Jellies and Marmalades."

(Control Method)

Principle

The sulfur dioxide solution is titrated directly against 0.05N iodine solution using starch as indicator. This control method is useful for determining the amount of sulfur dioxide in stock solutions or for estimating the sulfur dioxide content of fruit pulp preserved in this manner.

Reagents

1. Iodine solution–0.05N

   Dissolve 6.346 gm. iodine in a solution of 12 gm. potassium iodide in 100 ml. water and dilute to 1 liter.

2. Starch solution

   Mix 0.5 gm. soluble starch with a little cold water (about 15 ml.), pour into 100 ml. hot water and boil 1 to 2 minutes. Add enough NaCl to saturate, and store in a refrigerator, where it keeps for several weeks.

Procedure

With a 100-ml. pipette (inverted), remove about 100 ml. solution through the bunghole of the barrel. If the solution is not clear, filter through No. 4 Whatman paper into a 400-ml. beaker. Transfer the filtered solution to a 50-ml. burette as quickly as possible.

Pipette 10 ml. 0.05N iodine solution into a 500-ml. Erlenmeyer flask containing about 100 ml. water. Add 1 ml. starch solution. Titrate the SO_2 solution from the burette into the flask containing the iodine solution, rotating the flask frequently to keep the solution well mixed. When the color of the iodine solution becomes purple, add the solution from the burette dropwise, stopping at the point where one drop causes all color to disappear from the iodine solution.

Calculations

1 ml. 0.05N iodine reacts with 0.0016 gm. SO_2

$$\% SO_2 = \frac{\text{ml. iodine} \times \text{normality of iodine} \times 3.2}{\text{ml. } SO_2 \text{ solution required}}$$

$$\text{p.p.m. } SO_2 = \% SO_2 \times 10{,}000$$

References

Atkinson, F.E., and C.C. Strachan. Preservation of fruits with sulfur dioxide in British Columbia. Fruit Prod. J. 21: 5-8; 43-45; 60; 72-74; 110-112; 141-144; 153. 1941.

Wiegand, E. H. Process for the manufacture of maraschino cherries. Western Canner and Packer 29: 33-34. 1937.

MISCELLANEOUS PROCEDURES

CALCIUM

(Official Method)

Principle

Calcium is precipitated as calcium oxalate. The precipitate is dissolved in hot dilute sulfuric acid and titrated with standard potassium permanganate.

Ashing

Weigh duplicate 25-gm. blended samples into glazed procelain dishes. Evaporate to dryness on water bath or in forced-air oven at 100° and ash at low red heat (not to exceed 525°) until free of carbon particles.

Reagents

1. Methyl orange–0.05%
   Dissolve 0.05 gm. methyl orange in water and dilute to 100 ml.
2. Oxalic acid–2.5%
   Dissolve 12.5 gm. oxalic acid in water and dilute to 500 ml.
3. Sodium acetate–20%
   Dissolve 100 gm. sodium acetate in water and dilute to 500 ml.
4. Saturated ammonium oxalate solution. To 12.0 gm. $(NH_4)_2C_2O_4 . H_2O$ add 200 ml. of water.

Procedure

Dissolve the ash obtained above in 50 ml. of HCl (1 + 4) and heat for a few minutes. Be sure residue is acid. Filter through 15-cm. diameter No. 2 Whatman paper and wash thoroughly. Collect the washings in a 200-ml. volumetric flask. Make up to volume at 20°. To this filtrate or an aliquot, add 2 drops of methyl orange, and then ammonium hydroxide (1 + 4) drop by drop, until the solution is just alkali. Add dilute HCl (1 + 4) drop by drop until the solution is just acid. (When solution is cold and acid to the indicator, all the calcium phosphate is in solution. A small amount of phosphate or iron may remain undissolved at this point, but goes into solution when 0.5N acid is added.)

When the solution is just acid, add 10 ml. of 0.5N HCl and 10 ml. 2.5% oxalic acid. Heat the solution to the boiling point. Add 10 ml. of 20% solution of sodium acetate with constant stirring. Boil gently for 10 minutes. Add a few drops of saturated ammonium oxalate solution to make sure that all the calcium is precipitated. Hold overnight at 32 to 40° F.

Filter through 11-cm. diameter No. 40 Whatman paper into a beaker. Wash the precipitate free of chlorides with cold water (test with $AgNO_3$). Wash the precipitate into a 400-ml. beaker using hot water from wash bottle.

Keep the filter paper to add later in the titration. Make up to about 200 ml. with water, add 5 ml. conc. H_2SO_4 and heat to 70 to 80° C. Titrate hot with 0.1N $KMnO_4$ almost to completion (slight pink color). Add the filter paper in strips and complete titration to the first permanent pink color.

Calculations

1 ml. 0.1N $KMnO_4$ = 0.002 gm. calcium

References

Association of Official Agricultural Chemists. Official methods of analysis. 8th ed. p. 378. Washington, D.C. 1955.

Snell, D. F., and C.T. Snell. Colorimetric methods of analysis. Vol. II. 3rd ed. D. Van Nostrand Co., New York. 1949.

TANNIN AND COLORING MATTER

(Fruits and Fruit Products)

Principle

In a neutral solution, tannin and coloring matter react with permanganate and are measured by titration, using indigo solution as an indicator. As these solutions contain other oxidizable matter besides tannin, it is necessary to separate these using charcoal and titrating a second time to determine the quantity of permanganate actually required by the tannin present.

Reagents

See "Tannin and Coloring Matter," under "Wines and Cider."

Procedure

For light-colored products such as peach, apple and pear. use a blended 50-gm. sample; for deeper-colored products, use 25 gm.

Transfer the sample to 600-ml. beaker, add 300 ml. of water and boil gently for 1 hour, replacing the water lost by evaporation. Cool, transfer to 500-ml. volumetric flask and dilute to mark. Mix thoroughly and filter through No. 4 Whatman paper.

Pipette 400 ml. of filtrate into 600-ml. beaker, add 0.3 gm. powdered $CaCO_3$ and heat to boiling. Cool, transfer to 500-ml. volumetric flask and make up to volume. Mix thoroughly, and filter through No. 5 Whatman paper, refiltering if necessary until brilliantly clear.

Pipette 200 ml. filtrate into 2-liter procelain dish, add about 800 ml. of water and exactly 20 ml. of the indigo solution. Add standard $KMnO_4$ solution 1 ml. at a time, stirring vigorously until the blue color changes to green, then add a few drops at a time until the color becomes a golden yellow. Designate the ml. of $KMnO_4$ used as "a."

To the remaining filtrate add 1 gm. carbon and shake intermittently for 10 minutes. Filter through No. 5 Whatman paper, refiltering if necessary until clear. Pipette 200 ml. filtrate into the porcelain dish and add 800 ml. water and exactly 20 ml. of the indigo solution. Titrate with standard $KMnO_4$ in the manner described above. Designate the ml. of $KMnO_4$ solution required as "b."

Calculations

1 ml. 0.1N $KMnO_4$ = 0.0035 gm. tannin

(a - b) = ml. $KMnO_4$ solution required for oxidation of tannin

$$\% \text{ tannin} = \frac{(a - b) \times \text{normality of } KMnO_4 \times 3.5}{\text{wt. of sample titrated}}$$

References

Hartman, B. E. The polybasic acids of fruits and fruit products. Tannin and coloring matter. J. Assoc. Offic. Agr. Chemists 26: 452-462. 1943.

Strachan, C.C., A. W. Moyls, F.E. Atkinson and J. E. Britton. Chemical composition and nutritive value of British Columbia tree fruits. Canada Dept. Agr. Pub. 862. 1951.

ENZYME TESTS FOR ADEQUACY OF BLANCHING IN FROZEN VEGETABLES

Principle

This method is based upon measurement of the rate of color development in a guaiacol - hydrogen peroxide substrate under the catalytic influence of the enzyme present in the tissue.The reaction is brought about through the formation of an active peroxidase-peroxide complex, which oxidizes the colorless guaiacol directly to an orange-brown end product.

Reagents

1. Guaiacol solution—1%

   Dissolve 1 gm. or 0.9 ml. guaiacol in 50 ml. ethyl alcohol and add 50 ml. water.

2. Hydrogen peroxide—1%

   Dilute 1 part 3% H_2O_2 (free from preservatives) with 2 parts water.

Note: Glass dropping bottles of 100-ml. capacity are ideal containers. The reagents should be protected from light and stored in a refrigerator.

Testing reagents. The effectiveness of the reagents is determined by carrying out tests on two small pieces of fresh vegetables, one of which is boiled for 10 minutes and cooled. The fresh material should give a positive test, the heated one a negative test.

Preparing the Material

Select representative material from portions that were heated least in the blanching, i.e., the central portions of the thickest pieces. Use a stainless steel cutting knife.

For spinach, chard or similar leafy material, select a number of leaves and take the inch midrib portion beginning at the base of the leafy portion.

For asparagus spears, cut off and discard 3/4 inch from the butt end, then split the spears lengthwise.

For broccoli and cauliflower, split the stalk and head lengthwise.

For peas and other seed vegetables, cut each seed in half.

For string beans, cut 1/4- to 1/2-inch cross sections from a number of beans and split these cross sections lengthwise.

Procedure

Place the prepared material on a white porcelain saucer or evaporating dish. Add enough guaiacol solution to wet all of the cut surfaces, then immediately add a similar amount of hydrogen peroxide solution. At the end of 3 minutes note whether a reddish-brown color has developed. If none is observed the test for peroxidase is negative. Neglect any color that may develop after 3 minutes. The reactions, as read at the end of 3 minutes, are graded as follows:

negative	–	no color
trace	–	reddish-brown specks
faint	–	up to 25% of the material colored
heavy	–	material a solid reddish-brown color

References

Atkinson, F.E., C.C. Strachan and A. W. Moyls. B.C. Processor's Handbook. Fruit and Vegetable Processing Laboratory, Canada Dept. Agr. Experimental Farm, Summerland, B.C. Sept. 1947.

Joslyn, M. A. Report on peroxidase in frozen vegetables. J. Assoc. Offic. Agr. Chemists 36: 161-178. 1953.

CRUDE FAT OR ETHER EXTRACT

(For Fruit and Vegetable Products)

Principle

Fat-soluble material is extracted from an oven-dried sample using a Soxhlet extraction apparatus. The ether is evaporated and the remaining material weighed.

Procedure

Weigh 50-gm. blended sample into a 250-ml. beaker. Add about 75 ml. water and about 5 gm. asbestos. Mix and filter through No. 4 Whatman

filter paper. If globules of fat are present on the water layer, decant liquid into a separatory funnel and extract with several small portions of ether. If no fat is observed, liquid layer may be discarded. Keep the ether extract and combine it with sample before drying.

Place residue and filter paper in a thin aluminum foil dish and dry at 100° until moisture is removed, usually overnight.

Remove from oven and when cool, cut the dish and contents into small pieces and transfer directly into Soxhlet extraction thimble. Extract in the Soxhlet apparatus with anhydrous ether for at least 16 hours.

Remove thimble from the apparatus and distill off most of the ether by allowing it to collect in the Soxhlet tube and pouring it off when the tube is nearly full. When the ether has reached a small volume, pour it into a small beaker through a small funnel containing a plug of cotton. Rinse the flask and filter thoroughly, using several small portions of ether.

Evaporate the ether on a steam bath at low heat, preferably under a current of air. Dry at 100° for 1 hour, cool and weigh.

Calculations

$$\% \text{ crude fat} = \frac{\text{wt. of fat-soluble material} \times 100}{\text{wt. of sample}}$$

References

National Canners Association. Laboratory manual for the canning industry. Section 20, p. 32. National Canners Association, Washington, D. C. 1954.

ESTIMATION OF CALORIE CONTENT OF DIET FOODS

(Dietetic Fruit Spreads)

Take refractometer reading of sample at 20°. Multiply the reading by 4 to estimate number of calories per 100 gm.

If soluble solids reading is 21%:

Calories per 100 gm. = 21.0 × 4
= 84.

APPENDIX

STANDARD SOLUTIONS

ACIDS

Hydrochloric Acid–0.1N (3.646 gm. per liter)

Use conc. HCl (strength usually stated on bottle).

$\frac{3.646 \times 100 \text{ gm.}}{37.3}$ of 37.3% HCl gives 1 liter of 0.1N solution

Sp. gr. of conc. HCl = about 1.19

Therefore volume of conc. HCl required:

$\frac{3.646 \times 100}{37.3 \times 1.19}$ = 8.2 ml. per liter for 0.1N solution

Standardize against:

Standard 0.1N NaOH (titrate to pH 8.1)

Succinic Acid–0.1N $H_2C_4H_4O_4$ (5.9023 gm. per liter)

Dry 5 to 6 gm. pure succinic acid in open weighing bottle at 105° for about 10 hours; cool and store in desiccator. Weigh 2.9511 gm., transfer to 400-ml. beaker and dissolve in 150 to 200 ml. of water. Pour the solution into 500-ml. volumetric flask, rinsing out the beaker several times to insure complete transfer of the acid. Dilute to exactly 500 ml. and mix thoroughly. This prepares an exact 0.1N solution.

Sulfuric Acid–0.1N solution (4.904 gm. per liter)

Pour 3 ml. of conc. H_2SO_4 carefully into 10-12 ml. of water. Cool, mix thoroughly and dilute to 1 liter. Standardize by titration against standard NaOH or KOH to the phenolphthalein end point or to pH 8.1 with a pH meter.

Oxalic Acid–normal (63.023 gm. $H_2C_2O_4.2H_2O$ per liter)

Decinormal or less concentrated solutions are unstable and should be prepared fresh when needed. More concentrated solutions may deposit some of the acid when cooled to low temperatures but they are fairly stable at room temperature when protected from light.

BASES

Sodium Hydroxide—normal (40.005 gm. per liter)

Dissolve 42 gm. C.P. sodium hydroxide pellets in water and dilute to 1 liter in a volumetric flask.

Standardization of sodium hydroxide

1. For procedures using pH 8.1 as the end point: against weighed portions of succinic acid.

Dry succinic acid crystals at 105° for 8 hours and cool in desiccator. Take suitable portions of succinic acid (about 0.1 gm. for 0.1N NaOH and 0.05 gm. for 0.05N NaOH) and weigh accurately on an analytical balance. Dissolve in about 150 ml. water in 250-ml. beakers. Titrate in duplicate with NaOH solution to pH 8.1.

Calculations

$$\text{Normality of NaOH} = \frac{\text{wt. of succinic acid} \times 1{,}000}{\text{ml. NaOH} \times \text{equiv. wt. of acid}}$$

If 0.1047 gm. succinic acid required 17.1 ml. NaOH:

$$\text{Normality of NaOH} = \frac{0.1047 \times 1{,}000}{17.1 \times 59.03}$$

2. For procedures using bromophenol blue as indicator; against standard H_2SO_4.

Standardization of H_2SO_4 against Na_2CO_3

Heat Na_2CO_3 at 105° for 8 hours. Weigh out exactly 1.3250 gm., dissolve and make up to 250 ml. This makes exactly 0.1N Na_2CO_3 solution.

Pipette 5 ml. H_2SO_4 into 125-ml. Erlenmeyer flask. Add about 25 ml. water and 4 drops bromophenol blue. Titrate Na_2CO_3 from burette to a blue end point or pH 4.1, using a pH meter.

Standardization of NaOH against H_2SO_4

Pipette 5 ml. standardized 0.1N H_2SO_4 into 125-ml. Erlenmeyer flask. Add about 25 ml. water and 4 drops bromophenol blue indicator. Titrate NaOH from burette to color end point, or pH 4.1 using a pH meter.

For 0.05N NaOH use 2 ml. H_2SO_4.

OXIDIZING AND REDUCING SOLUTIONS

Potassium Dichromate—0.1N (4.9037 gm. per liter)

Dry crystals at 120 to 140° for 2 to 4 hours. Cool in a desiccator and weigh 5.0 gm. to the nearest milligram. Dissolve in about 200 ml. water and transfer to 1-liter volumetric flask, dilute to volume and mix thoroughly.

$$\text{Normality} = \frac{\text{wt. of potassium dichromate}}{49.037}$$

Potassium Permanganate–0.1N (3.1606 gm. per liter)

Dissolve 3.3 gm. of dry $KMnO_4$ in about 200 ml. water and transfer to a 1-liter flask. Make up to volume at 20°.

Standardization with sodium oxalate

Weigh out accurately three 0.25– to 0.30-gm. samples of sodium oxalate having an assay value of 99.95%, transfer each portion to a 600-ml. beaker, using 250 ml. dilute sulfuric acid (5 + 95). Stir until the oxalate has dissolved, then add rapidly from a burette about 95% of the amount of permanganate needed for complete oxidation of the sample. Allow the solution to stand until the permanganate is decolorized, then heat to 55 to 60°. Complete the titration at this temperature, stirring gently and allowing each drop to become decolorized before adding the next; titrate to first permanent pink.

$$\text{Normality of } KMnO_4 = \frac{\text{gm. sodium oxalate} \times 1{,}000}{\text{ml. of } KMnO_4 \times 67.0}$$

Sodium Thiosulfate–0.1N (24.8192 gm. $Na_2S_2O_3 \cdot 5H_2O$ per liter)

Weigh 25.0 gm., dissolve in 200 ml. water, transfer to 1-liter flask and make up to volume. Mix the solution thoroughly, allow it to stand for a few days, and then siphon off the clear liquid. The solution is standardized indirectly with potassium dichromate.

Standardization with potassium dichromate

Accurately weigh 0.20- to 0.23-gm. $K_2Cr_2O_7$ (dried 2 hours at 105°). Transfer to 250-ml. beaker using about 150 ml. water. Add 2 gm. potassium iodide and mix. Add 20 ml. 1N HCl, swirl, and let stand for 10 minutes. Start titrating with the sodium thiosulfate from burette, adding about 80% of the required amount. Add 1 ml. starch and complete titration to point where solution changes from blue-green to light green.

$$\text{Normality} = \frac{\text{gm. } K_2Cr_2O_7 \times 1000}{\text{ml. } Na_2S_2O_3 \times 49.037}$$

Iodine–0.1N (12.693 gm. per liter)

Dissolve 13.5 gm. pure resublimed iodine in a solution of 24 gm. potassium iodide in 200 ml. of H_2O and dilute to 1 liter. The solution is standardized by titrating against a known volume of standard thiosulfate, with a few drops of starch solution as indicator.

INDICATORS

Phenolphthalein–pH range 8.3 to 10

Dissolve 1 gm. in 100 ml. neutral ethyl alcohol and water. Use 1 drop per 100 ml. solution.

Methyl red—pH range 4.4 to 6.0

Dissolve 1 gm. in 100 ml. 95% ethyl alcohol. This indicator is easily reduced with loss of color, and readings must be made shortly after it is added to the solution.

Methyl orange—pH range 2.9 to 4.0

Dissolve 0.5 gm. in 1000 ml. water.

Bromophenol blue—pH range 3.0 to 4.6

Dissolve 0.1 gm. in 25 ml. water and dilute to 100 ml. with water.

Starch solution—0.5%

Dissolve 0.5 gm. soluble starch in about 15 ml. cold water and pour into 100 ml. hot water. Boil 1 to 2 minutes.

Cleaning solution

Sodium or potassium dichromate (commercial)	40 gm.
Water	150 ml.

Dissolve with a little heat if necessary, then cool to room temperature and add slowly 230 ml. conc. sulfuric acid (tech.).

Note: As a precaution this solution should always be prepared over a sink.

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