Method 8.0

Starch (Direct Acid Hydrolysis)

Purpose: To determine, as starch, the carbohydrate materials which are hydrolysed to reducing sugars by hydrochloric acid. (Not applicable to products with added sugars).

A. Apparatus:

1. Erlenmeyer flask 500 mL, with T.S. 24/40 ground joint.
2. West type condenser, 400 mm in length, with water-cooled drip tip T.S. 24/40 ground joint.
3. Volumetric flask, 250 mL, with ground glass stopper.
4. Gooch crucible.
5. Pipettes, transfer type, 5, 25 and 50 mL.
6. Buret, 50 mL, graduated in 0.1 mL.

B. Reagents:

1. Hydrochloric acid solution, Sp. gr. 1.125 - Dilute 680 mL of 37% HCL by wt. (Sp. gr. = 1.19 at 20°C.) to 1 liter.
2. Sodium hydroxide solution, ca. 2.5N, (10% w/v).
3. Ethyl ether, ACS grade, anhydrous.
4. Ethyl alcohol solution, 10% by volume.
5. Ethyl alcohol absolute, ACS grade.
6. Ceramic Fiber - prepared as directed under Method 7.0.

Method 8.0

Starch (Direct Acid Hydrolysis)

7. Modified Fehling's solution:
   (a) Copper sulfate solution--Dissolve 34.639 g of copper sulfate ($CuSO_4.5H_2O$) in distilled water, dilute to 500 mL and filter through prepared ceramic fiber.
   (b) Alkaline tartrate solution--Dissolve 173 g of sodium tartrate, $NaKC_4H_4O_6.4H_2O$ (Rochele salt) and 50 g of NaOH in distilled water. Dilute to 500 mL, allow to stand for 2 days and filter through prepared ceramic fiber.

8. Indicator paper (universal).

C. Preparation of Sample:

1. Use samples prepared as directed under Method 1.0.

D. Procedure:

1. Weigh 4 g to the nearest 0.01 g of sample and transfer to a funnel containing a Whatman No. 2 filter paper or equivalent. Extract the sample with 5 successive 10 mL portions of ethyl ether. Allow the ether to evaporate from the residue, wash with 150 mL of the 10% alcohol solution, and then with 15 to 20 mL of absolute ethyl alcohol.

2. Carefully transfer the insoluble residue from the filter paper to the 500 mL Erlenmeyer flask with water, using a wash bottle and gently rubbing the paper with a rubber policeman. Add distilled water to make the total volume 200 mL and then add 20 mL of the HCl solution (Sp. gr 1.125). Connect the flask to a reflux condenser and boil for 2.5 hours.

3. Cool and add 2.5N NaOH solution slowly with stirring until the solution is almost neutral to indicator paper (pH 6-7). Solution must not be alkaline at any time. Transfer to the 500 mL volumetric flask, make to volume at room temperature and mix well.

4. To determine the amount of reducing sugars, filter the hydrolysate through a dry filter paper, discarding the first 10 mL portion of filtrate. Pipette 25 mL each of the copper sulfate and alkaline tartrate solutions into a 400 mL beaker and add an aliquot (See Note 1) of the filtered sample solution. If the aliquot is less than 50 mL, add distilled water to make the final volume 100 mL.

5. Cover the beaker with a watch glass and heat on an iron-wire gauze over a Bunsen burner or on a hot plate. The burner or hot plate must be pre-set to bring the solution to a boil in exactly 4 minutes. Continue boiling exactly 2 minutes.

6. Filter the hot solution immediately through the prepared, tared Gooch crucible with the aid of suction. Wash the precipitated Cu_2O thoroughly with water at ca. 60° C. Then wash the precipitate with 10 mL of absolute alcohol and finally with 10 mL of ether. Dry the precipitate 30 minutes in an oven at 110°±2° C, cool to room temperature in a desiccator and weigh accurately. (Note 2 & 3)

E. Calculation:

1. Refer to the standard Munson and Walker table to find the mg of dextrose corresponding to the weight of Cu_2O found.

2. Dextrose, % = $\dfrac{\text{Wt. of dextrose (mg) x 500 x 0.1}}{\text{Wt. of sample (g) x aliquot (mL)}}$

3. Starch, % = % dextrose x 0.90.

F. Statistics:

TBD

G. Notes:

1. The optimum aliquot depends on the starch content of the sample being analyzed. The aliquot should contain between 100 to 200 mg of dextrose.

Expected starch content	aliquot in mL
60	25
50	35
40	50
30	50
20	50

Method 7.0

Crude Fiber

Purpose: To determine, as crude fiber, the organic matter in the dried residue remaining after digesting the sample with dilute sulfuric acid and sodium hydroxide.

A. Apparatus:

1. Condenser that will maintain a constant volume of solution throughout the digestion period. An Allihn condenser with T.S. 45/50 ground joint is recommended.

2. Digestion flask of such a size and shape that the solution will be not less than 1 inch (25 mm.) nor more than 1.5 inches (38 mm.) in depth. A one liter Erlenmeyer flask with 45/50 ground joint is recommended.

3. California Modified Buchner Funnel (Labconco Catalog #55100). Alternatively a filter cloth, of such character that no appreciable solid matter can pass through it during rapid filtration, may be used. Retention may be tested by running filtrate through a Gooch crucible. Butcher's linen, dress linen with ca. 45 threads to an inch, or No. 40 filter cloth made by the National Filter Media Corporation, Hamden, Connecticut 06514, or equivalent may beused. (Note 1)

4. Electric muffle furnace or a Meeker-type burner.

5. Desiccator containing an efficient desiccant.

6. Gooch crucible--Pour the prepared ceramic fiber into Gooch crucible to make a mat of such thickness that the holes are barely visible (when looking through the crucible at a light source) and ignite.

7. Oven, preferably circulating air type.

B. Reagents:

1. Sulfuric acid solution, 0.255N, (12.5 g of concentrate H_2SO_4, Sp. gr. 1.84, dilute to 1 liter). (Note 2)

2. Sodium hydroxide solution, 0.312N, (12.5 g carbonate free NaOH pellets per liter). (Note 2)

3. Prepared ceramic fiber, Place 60g ceramic fiber (Cerafiber, 8 lb/cu ft (ft^3), E.J. Bartell Co., 700 Powell Ave, S.W., Renton, WA 98055) in blender, add 800 mL H_2O, and blend 1 min at low speed. Det. blank by treating ca. 2 g (dry wt) of prepd ceramic fiber with acid and alkali as in detn. Correct crude fiber results for any blank, which should be negligible (ca. 2 mg).

4. Ethyl alcohol, 95%, ACS grade.

5. Methylene chloride, anhydrous (dichloromethane), ACS grade.

6. Litmus Paper - blue.

C. Preparation of Sample:

1. Use sample prepared as directed under Method 1.0.

D. Procedure:

1. Extract 2 g of sample with methylene chloride or use the fat free residue from method 11.0. Transfer the residue together with ca. 0.5 g of ceramic fiber to the digestion flask.

2. Add 200 mL of the H_2SO_4 solution, connect the digestion flask to the condenser and place on a preheated hot plate or digestion rack adjusted so that the acid will boil in ca. 5 minutes. Continue boiling briskly for exactly 28 minutes with frequent rotation of the flask to insure through wetting and mixing of the sample. Material should not be allowed to remain on the sides of the flask out of contact with the solution. A blast of air directed into the flask from a glass tube inserted through the condenser will serve to reduce frothing. Successive sample digestions should be started at ca. 3 minute intervals to facilitate accurate timing.

3. After boiling 28 minutes, remove the flask and filter immediately through the California Modified Buchner funnel or through a filter cloth in a fluted funnel using a suction flask to speed filtration. Wash with boiling water until washings are no longer acid. Check alkalitity using litmus paper.

4. Transfer the sample and ceramic fiber quantitatively to the digestion flask, washing the filter cloth or Buchner funnel with 200 mL of the NaOH solution. A wash bottle marked to deliver 200 mL is convenient.

5. Connect the flask to the reflux condenser, place on the preheated hot plate or digestion rack, bring to a boil in ca. 5 minutes, and boil exactly 28 minutes. Successive sample digestion's should be started at ca. 3 minute intervals to facilitate accurate timing.

6. After 28 minutes, remove the flask and immediately filter through a Gooch crucible.

7. Wash the residue thoroughly with water and then with ca. 15 mL of ethyl alcohol.

8. Dry the crucible and contents at 110° ±2° C. to a constant weight (ca. one hour). Cool in a desiccator and weigh.

9. Ignite the crucible and contents in an electric muffle furnace at ca. 600° C. or over a Meeker burner at dull red heat for ca. 20 minutes. Cool in a desiccator and weigh. Determine the loss in weight on ignition.

E. Calculation:

$$\text{Crude fiber \%} = \frac{\text{Loss in weight on ignition (g)}}{\text{Wt. of original sample (g)}} \text{ X } 100$$

F. Statistics:

TBD

G. Notes:

1. Finely ground materials tend to yield low values, and the filtration may be slow and difficult.

2. The concentration of both acid and alkali solutions should be checked by titration. If the concentration differs by more than ±0.01 N from the nominal values adjust to within this range.

G.References:

N/A

2. Conduct a blank determination using 50 mL of the reagent and 50 mL of water. If the weight of the Cu_2O obtained exceeds 0.5 mg, correct the result of the determination accordingly. The alkaline tartrate solution deteriorates on standing and the quantity of Cu_2O obtained in the blank determination increases.

3. The procedure is very empirical and needs to be followed exactly.

H. References:

N/A