AOAC Official Method 940.30
Starch in Prepared Mustard

First Action 1940
Final Action

A. Reagents

(**a**) *Calcium chloride solution.*—30 g/100 mL solution adjusted to 0.01M alkalinity.

(**b**) *Alcoholic sodium hydroxide solution.*—Mix 70 mL alcohol with 30 mL 0.1M NaOH.

(**c**) *Iodine–potassium iodide solution.*—Dissolve 2 g I_2 and 6 g KI in 100 mL H_2O.

B. Determination

Place 5 g prepared mustard in 500 mL Erlenmeyer and pipet in 100 mL $CaCl_2$ solution, swirling flask gently until all lumps are broken. Add calculated volume 1M NaOH to neutralize acid in weight prepared mustard taken for analysis. Add glass beads. Connect to reflux condenser, first wetting inside of condenser and stopper with H_2O and draining 1 min. Heat gently (on hole heating mantle) to avoid initial foaming, and boil 15 min.

Leaving condenser connected, cool flask to room temperature in pan of cold H_2O. Remove flask, stopper, and shake vigorously. Pour contents into centrifuge bottle and centrifuge 5 min at 1500 rpm. Withdraw as much as possible of partially clarified middle layer (ca 75 mL) and filter through 11 cm circle of absorbent cotton ca 5 cm thick placed in 60° funnel. Pipet 50 mL filtrate into second centrifuge bottle containing 150 mL alcohol, stopper, and shake vigorously several minutes. Centrifuge at 1500 rpm until clear (ca 5 min).

Decant liquid through glass fiber filter in Caldwell crucible, using suction, without transferring starch to crucible. Transfer pad to same centrifuge bottle, and rinse all particles adhering to crucible into bottle with H_2O. Add several glass beads and H_2O to ca 100 mL. Stopper and shake vigorously until precipitate is as finely dispersed as possible. Add slight excess I_2–KI solution (2–3 mL) and 30 mL saturated $(NH_4)_2SO_4$ solution. Stopper and shake bottle. Rinse particles adhering to stopper into bottle, and centrifuge until clear.

Decant supernate, with suction, through glass fiber filter in Caldwell crucible. Add 50 mL alcoholic NaOH solution to precipitate in centrifuge bottle. Stopper and shake vigorously. Wash stopper with 70% alcohol. Centrifuge and decant supernate through same pad as before. Repeat treatment with the NaOH solution until practically all blue disappears (usually 2–3 treatments). Without centrifuging, transfer contents of bottle to Caldwell crucible, using 70% alcohol. Aspirate until pad is dry; then transfer pad to 500 mL Kjeldahl flask. Rinse bottle and crucible with 10 mL HCl (specific gravity 1.1029) followed by five 10 mL portions H_2O, carefully removing all adhering particles. Attach Kjeldahl flask to reflux condenser, first adding glass beads to lessen bumping. Place on asbestos board with center hole and boil 1 h. Cool, neutralize with NaOH (1 + 1) (methyl orange), and filter into 200 mL volumetric flask; rinse flask and filter thoroughly, and dilute to volume with H_2O. Mix well, and determine glucose in 50 mL aliquot by **906.03★B** (*see* 44.1.16). (Blank on Fehling solution should be ≤0.3 mg.)

C. Calculation

$$\text{Starch, \%} = \frac{\text{g glucose} \times 0.9(100 + V + W) \times 8}{\text{weight test portion}}$$

where V = mL 1M NaOH used to neutralize acidity, **920.174** (*see* 43.1.23), and W = g H_2O in test portion taken [calculated from solids, **920.171** (*see* 43.1.19)].

References: *JAOAC* **23**, 579(1940); **24**, 700(1941);
25, 97, 705(1942).

Revised: March 1996