Rev 3 Stage 4

E43 WHEAT STARCH

Tritici amylum

DEFINITION

Wheat starch is obtained from the caryopsis of *Triticum aestivum* L. (*T. vulgare* Vill.).

IDENTIFICATION

A. Examined under a microscope using equal volumes of glycerol and water, it presents large and small granules, and, very rarely, intermediate sizes. The large granules, usually 10 µm to 60 µm in diameter, are discoid or, more rarely, reniform when seen face-on. The central hilum and striations are invisible or barely visible and the granules sometimes show cracks on the edges. Seen in profile, the granules are elliptical and fusiform and the hilum appears as a slit along the main axis. The small granules, rounded or polyhedral, are 2 µm to 10 µm in diameter. Between orthogonally oriented polarizing plates or prisms, the granules show a distinct black cross intersecting at the hilum.

B. Suspend 1 g in 50 ml of water, boil for 1 min and cool. A thin, cloudy mucilage is formed.

C. To 1 ml of the mucilage obtained in identification test B, add 0.05 ml of *iodine solution*. A dark-blue color is produced which disappears on heating.

TESTS

pH. Shake 5.0 g with 25.0 ml of freshly boiled and cooled water for 60 s. Allow to stand for 15 min. The pH of the solution is 4.5 to 7.0.

Iron. Shake 1.5 g with 15 ml of dilute hydrochloric acid (1 in 5). Filter. The filtrate complies with the limit test for iron (10 ppm).

Procedure — To 10 ml of the filtrate, add 2 ml of a 200 g/l solution of citric acid and 0.1 ml of thioglycollic acid. Mix, make alkaline with ammonia and dilute to 20 ml with water. Prepare a standard in the same manner, using 10 ml of iron standard solution (1 ppm Fe). After 5 min, any pink colour in the test solution is not more intense than in the standard.

Total protein. Not more than 0.3 per cent of total protein (corresponding to 0.048 per cent of nitrogen, conversion factor: 6.25), determined by sulfuric acid digestion.

Procedure — Carry out a blank determination by placing 4 g of a powdered mixture of 100 g of *dipotassium sulfate*, 3 g of *copper sulfate* and 3 g of *titanium dioxide* and three glass beads in a combustion flask. Wash any adhering particles from the neck into the flask with 25 ml of *sulfuric acid*, allowing it to run down the sides of the flask, and mix the contents by rotation. Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulfuric acid. Heat gradually at first, then increase the temperature until there is

vigorous boiling with condensation of sulfuric acid in the neck of the flask; precautions should be taken to prevent the upper part of the flask from becoming overheated. Continue the heating until a clear solution is obtained. Cool, dissolve the solid material by cautiously adding to the mixture 25 ml of *water*, cool again and place in a steam-distillation apparatus. Add a volume of *strong sodium hydroxide solution* suitable to make the colour of the solution turn from bluish-green to brown or black and distil immediately by passing steam through the mixture. Collect about 40 ml of distillate in 50.0 ml of *0.01 M hydrochloric acid* and enough *water* to cover the tip of the condenser. Towards the end of the distillation, lower the receiver so that the tip of the condenser is above the surface of the acid. Take precautions to prevent any water on the outer surface of the condenser from reaching the contents of the receiver. Titrate the distillate with *0.025 M sodium hydroxide*, using *methyl red mixed solution* as indicator (n_1 ml of *0.025 M sodium hydroxide*).

Repeat the test placing first 3.0 g (*m* g) of the substance to be examined in the combustion flask, using the same volume of *strong sodium hydroxide solution* and titrating the distillate as for the blank determination (n_2 ml of *0.025 M sodium hydroxide*).

$$\text{Content of nitrogen (per cent)} = [0.03503\ (n_1 - n_2)] / m$$

Oxidising substances. Transfer 4.0 g to a glass-stoppered, 125 ml conical flask and add 50.0 ml of water. Insert the stopper and swirl for 5 min. Transfer to a glass-stoppered 50 ml centrifuge tube and centrifuge. Transfer 30.0 ml of the clear supernatant liquid to a glass-stoppered 125 ml conical flask. Add 1 ml of glacial acetic acid and 0.5 g to 1.0 g of potassium iodide. Insert the stopper, swirl, and allow to stand for 25 min to 30 min in the dark. Add 1 ml of *starch solution* and titrate with 0.002 M sodium thiosulfate until the starch-iodine colour disappears. Carry out a blank determination. Not more than 1.4 ml of 0.002 M sodium thiosulfate is required (0.002 per cent, calculated as H_2O_2).

1 ml of 0.002 M sodium thiosulfate is equivalent to 34 µg of oxidising substances, calculated as hydrogen peroxide.

Sulfur dioxide. Not more than 50 ppm.

Procedure — Introduce 150 ml of water into the flask (A) (see Figure —) and pass carbon dioxide through the whole system for 15 min at a rate of 100 ± 5 ml/min. To 10 ml of dilute hydrogen peroxide solution (1 in 10) add 0.15 ml of a 1 g/l solution of bromophenol blue in alcohol (20 per cent V/V). Add 0.1 M sodium hydroxide until a violet-blue colour is obtained, without exceeding the end-point. Place the solution in the test-tube (D). Without interrupting the stream of carbon dioxide, remove the funnel (B) and introduce through the opening into the flask (A) 25.0 g (*m* g) of the substance to be examined with the aid of 100 ml of water. Close the tap of the funnel and add 80 ml of dilute hydrochloric acid (1 in 5) to the funnel. Open the tap of the funnel to permit the hydrochloric acid solution to flow into the flask, guarding against the escape of sulfur dioxide into the funnel by closing the tap before the last few mL of hydrochloric acid solution drain out. Boil for 1 h. Open the tap of the funnel and stop the flow of carbon dioxide and also the heating and the cooling water. Transfer the contents of the test-tube with the aid of a little water to a 200 ml wide-necked, conical flask. Heat on a water-bath for 15 min and allow to

cool. Add 0.1 ml of a 1 g/l solution of bromophenol blue in alcohol (20 per cent V/V) and titrate with 0.1 M sodium hydroxide until the colour changes from yellow to violet-blue (V_1 ml). Carry out a blank titration (V_2 ml). Calculate the content of sulfur dioxide in parts per million from the expression:

$$32\,030 \times (V_1 - V_2) \times (n/m)$$

n = molarity of the sodium hydroxide solution used as titrant.

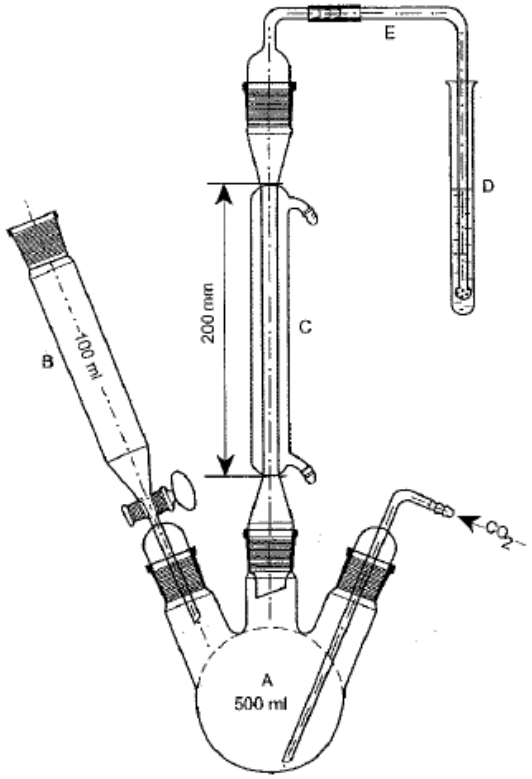

Figure — *Apparatus for the determination of sulfur dioxide*

Loss on drying. Not more than 15.0 per cent, determined on 1.000 g by drying in an oven at 130 ℃ for 90 min.

Sulfated ash. Not more than 0.6 per cent, determined on 1.0 g.

Microbial contamination (internationally harmonized methods) - TAMC: acceptance criterion 10^3 CFU/g. TYMC: acceptance criterion 10^2 CFU/g. Absence of Escherichia coli.

REAGENTS

Iodine solution — To 10.0 ml of 0.05 M iodine solution, add 0.6 g of potassium iodide and dilute to 100.0 ml with water. Prepare immediately before use.

Strong Sodium hydroxide solution — Dissolve 42 g of sodium hydroxide in water and dilute to 100 ml with the same solvent.

Methyl red mixed solution — Dissolve 0.1 g of methyl red and 50 mg of methylene blue in 100 ml of alcohol. Colour change: pH 5.2 (red-violet) to pH 5.6 (green).

Starch solution — Triturate 1.0 g of soluble starch with 5 ml of water and whilst stirring pour the mixture into 100 ml of boiling water containing 10 mg of mercuric iodide.

Carry out the test for sensitivity each time the reagent is used.

Test for sensitivity. To a mixture of 1 ml of the *starch solution* and 20 ml of water, add about 50 mg of potassium iodide and 0.05 ml of *iodine solution*; the solution is blue.