AMYLOSE (Blue Value)

PRINCIPLE

Iodine complexes preferentially with the amylose (linear fraction) in corn starch. The starch sample (defatting is optional) is dispersed in alkali and neutralized. An excess of standard iodine solution is added and the resulting stable blue complex is measured spectrophotometrically. Amylose content is estimated by reference to a calibration plot, prepared with the aid of defatted starches having known amylose contents (Note 1).

SCOPE

This method is applicable to unmodified corn starches.

SPECIAL APPARATUS

Spectrophotometer: An instrument having a continuously-variable wavelength control in the visible region, with matching 1.0 cm cuvets, is recommended.

REAGENTS

1. Iodine Solution, 0.20%: Dissolve 20.0 g of reagent grade potassium iodide (KI) in 200 mL of purified water in a 1 L volumetric flask. Add 2.000 g of resublimed iodine (I_2) and stir. When the iodine is dissolved completely, dilute to volume with purified water and mix. Store in a nonactinic bottle.

   Iodine solution prepared in this manner will contain exactly 2.00 mg iodine per mL and is stable for long periods. Standardize the solution occasionally against National Institute of Standards and Technology arsenious oxide. When iodine concentration falls below 1.98 mg per mL, discard and prepare fresh reagent.

2. Sodium Hydroxide Solution, 1 *N*: Standard

3. Hydrochloric Acid Solution, 0.1 *N*: Standard

4. Ethyl Alcohol: Absolute

5. Phenolphthalein Indicator, 0.1%

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AMYLOSE (Blue Value) — continued

PROCEDURE

Sample Analysis: Grind sample to eliminate hard or coarse particles, blend and determine moisture content by an approved method. Defatted samples usually provide more accurate data, and defatting, if desired, can be accomplished rapidly and efficently by extracting with a mixed solvent composed of methanol and dimethylsulfoxide (Note 2).

Weigh a starch sample containing 100.0 ± 0.1 mg of dry substance and transfer quantitatively to a 100 mL volumetric flask. Add 1 mL of ethyl alcohol and swirl to disperse the ethanol. Add 10.0 mL of 1 *N* sodium hydroxide solution and again swirl to disperse sample. Allow dispersion to stand until sample is completely gelatinized (about 1 hr.). The mixture must be smooth and free of lumps. Dilute to volume with purified water and mix.

Pipet 2.50 mL of this solution into a 100 mL volumetric flask and add 50 mL of purified water. Add 2 drops of phenolphthalein indicator and titrate with 0.1 *N* hydrochloric acid solution from a buret until the pink indicator color just disappears. Add 2.0 mL of 0.20% iodine solution, dilute to volume with purified water, mix and let stand 30 mins.

Prepare a reference solution (blank) by diluting 2.0 mL of 0.20% iodine solution to 100 mL volume with purified water and mix before use. Fill one of two 1 cm matching cuvets with the reference solution and fill the other with the sample solution. Determine absorbance of sample solution at 620 nm against the reference solution.

Standardization: Calibration data are obtained by similar analysis of defatted, unmodified starches. Amylose contents of the starches used for standardization should range from 0% (waxy maize) to 75% (high-amylose maize) as determined by Method B-28, Iodine Affinity, in this manual. Defatting the standards may be accomplished either according to the procedure described in Method B-28 or by the methanol-dimethylsulfoxide mixed solvent procedure described in Note 2.

Process the defatted starch standards by the procedure described earlier under "Sample Analysis." Determine absorbance of the standard sample solutions against the reference solution at 620 nm in 1.0 cm matching cuvets. Plot absorbance versus amylose content for the starch standards.

AMYLOSE (Blue Value) — continued

CALCULATION

Defatted Samples:

Amylose Content, % = % Amylose (From Graph)

Nondefatted Samples (Note 3):

Amylose Content, % = Amylose (From Graph $\times$ 1.06

NOTES AND PRECAUTIONS

1. This method is similar to that described by G. A. Gilbert and S. P. Spragg, *Methods in Carbohydrate Chemistry*, Vol. 4, p. 168 (R. L. Whistler, Ed., Academic Press, New York, 1964).

2. Samples and standard starches may be defatted by the following procedure. Place 10 to 11 g of starch sample in a 250 mL Erlenmeyer flask equipped with a ST 24/40 round glass joint. Insert a stirring bar, add 30 mL of anhydrous methanol and 20 mL of anhydrous dimethylsulfoxide. Place flask on a magnetic stirring hot plate and attach a water-cooled condenser. Stir at a moderate rate; bring sample-solvent mixture to reflux temperature and reflux for 30 mins.

   Transfer hot mixture to medium-porosity, sintered-glass Büchner funnel and recover starch by vacuum filtration. Rinse flask into funnel with anhydrous methanol from a wash bottle, and wash starch with an additional 50 mL of anhydrous methanol; maintain vacuum until excess solvent is removed.

   Reslurry filter cake in hot anhydrous methanol and repeat washing step above.

   Transfer extracted starch sample to a watch glass or Petri dish and at room temperature air-dry overnight.

3. The correction factor, 1.06, is a typical value. Laboratories wishing to apply this method to nondefatted samples should develop their own correction factor.