APPENDIX

(ANALYTICAL METHODS)

1. Starch content (Glucoamylase method)

(Association of Official Analytical Chemists, 1997)

Reagents

1) Glucoamylase

2) 4M Acetate buffer, pH 4.8

Procedure

1) Grind sample to particle size smaller than 0.5 mm, which is equivalent to 40-mesh stainless-steel sieve.

2) Determine moisture content in ground grain by an AACC approved method to correct starch data to dry-weight basis.

3) Into dried and tared Erlenmeyer flask, weigh sample not more than 1.0 g (generally 0.5 g) and containing less than 0.5 g starch.

4) Add 25 ml water with stirring to disperse product and adjust pH to between 5 and 7, if necessary. Boil suspension with gentle stirring for 3 min, then pressure heat at 135° for 1 hr.

5) Remove from autoclave, maintain temperature near 55°, and add 2.5 ml acetate buffer and sufficient water to adjust total weight of solution to 45 ± 1 g.

6) Immerse Erlenmeyer flask in water bath with shaker at optimal temperature of glucoamylase used (glucoamylase extract from *Rhizopus delemar* 55 ± 1°) and add 5 ml glucoamylase solution.

7) Hydrolyze 2 hr with continuous shaking, filter through folded filter paper into 250-ml volumetric flask, wash quantitatively, and dilute to volume.

8) Transfer 1-ml aliquots containing 20–60 mg D-glucose to test tubes. To obtain this range of glucose concentrations, it may be necessary to dilute hydrolysate of step 7.

9) Add 2 ml enzyme-buffer-chromogen mixture, shake tubes, and place in dark at 37 ± 1° exactly 30 min to develop color.

10) Stop reaction with 2 ml 18N H_2SO_4 and measure absorbance at 540 nm.

11) Prepare standard D-glucose curve from 0 to 60 mg/ml and blank for each series of analyses.

Calculation

$$\%\,Starch = 0.9 \times \frac{M}{10^6} \times \frac{V_1}{1} \times \frac{250}{V_0} \times \frac{100}{E} \times \frac{100}{MS} = 2.25 \times \frac{M \times V_1}{V_0 \times E \times MS}$$

in which E = weight in grams of sample, M = weight in mg of D-glucose obtained from standard curve, V_o = volume in milliliters of aliquot from 250-ml flask, MS = percentage dry weight of sample, V_1 = volume in milliliters if extra dilution is done in step 8. Value of V_1 is 1.0 when no extra dilution is done.

2. Crude fiber content

(Association of Official Analytical Chemists, 1984)

Reagents

1) Sodium Hydroxide Solution, 0.312 N (1.25%):

2) Sulfuric Acid Solution, 0.255 N (1.25%):

3) Alcohol: Methyl, isopropyl, or 95% ethyl alcohol

4) Antifoam: Dow Corning Antifoam A Emulsion diluted 1 + 4 with water

Procedure

Grind about 50 g of sample through a laboratory cutting mill to 20 mesh or finer, and mix thoroughly. Determine moisture content of the ground sample by the "Standard" toluene distillation method, or alternate procedure giving equivalent results.

Weigh accurately about 2 g of sample and transfer to a 9 cm hard filter paper supported on a filter cone in a 60° funnel. Extract with three 25 mL portions of ether and apply vacuum until sample is dry. Transfer extracted sample quantitatively by brushing into a 600 mL beaker of the fiber digestion apparatus. Add 20 mL of well-mixed ceramic fiber suspension (containing about 1.5 g of fiber - dry weight), 200 mL of boiling 1.25% sulfuric acid solution, and 1 drop of diluted antifoam. Place beaker on digestion apparatus with preadjusted heater and boil exactly 30 minutes, rotating beaker periodically to keep solids from adhering to sides.

Remove beaker and filter contents through California Buchner funnel precoated with about 0.75 g of ceramic fiber - dry weight; rinse beaker with 50-75 mL of boiling water, and wash through funnel. Repeat with three 50 mL portions of water, and suck dry. Return fiber mat with residue to beaker by blowing back through funnel. Add 200 mL of boiling 1.25% sodium hydroxide solution, return to heater and boil exactly 30 minutes. Remove beaker and filter as before. Wash with 25 mL of boiling 1.25% sulfuric acid solution, three 50 mL portions of water, and 25 mL of alcohol. Remove mat and residue, and transfer to ashing dish.

Dry fiber mat and residue at 130 ± 2 °C for 2 hours. Cool in a desiccator and weigh. Ignite at 600 ± 15 °C to constant weight (30 minutes usually sufficient). Cool in desiccator and weigh.

Run a blank determination on the prepared ceramic fiber using the same quantity of fiber and the same amounts of acid and alkali as in the determination.

Calculation

$$\% \text{ Crude Fiber (dry basis)} = \frac{((\text{Dry Residue Wt. (g)} \times \text{Ignited Residue Wt. (g)} \times \text{Blank Wt. Loss (g)})) \times 100 \times 100}{\text{Sample Wt. (g)} \times \text{Sample Moisture (\%)}}$$

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