Food and Agriculture Organization of the United Nations

World Health Organization

Analytical Method prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 82nd meeting 2016

PURITY TESTS FOR MODIFIED STARCHES (Vol 4)

This analytical method was also published in: *Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 82nd meeting 2016. FAO JECFA Monographs 19*

PURITY TESTS FOR MODIFIED STARCHES

Carboxyl groups

Principle

The carboxyl containing starch is equilibrated with mineral acid to convert carboxyl salts to the acid form. Cations and excess acid are removed by washing with water. The washed sample is gelatinized in water and titrated with standard alkali.

NOTE: Native phosphate groups present in potato starch increase the titre found in this method (See NOTE 6).

Reagents

Hydrochloric Acid Solution, 0.10 N: Standardization unnecessary
Sodium Hydroxide Solution, 0.10 N : Standardized
Phenolphthalein Indicator, 1%

Procedure

If necessary, grind sample completely through a laboratory cutting mill to 20 mesh or finer, taking precautions to prevent any significant change in moisture, and mix thoroughly.

Weigh accurately a sample containing not more than 0.25 milliequivalents of carboxyl (Note 1), and transfer quantitatively to a 150-ml beaker. Add 25 ml of 0.1 N hydrochloric acid and stir occasionally over a period of 30 min. Vacuum filter the slurry through a medium porosity fritted-glass crucible or small funnel, using a fine stream of water from a wash bottle to aid quantitative transfer of the sample. Wash the sample with distilled water (300 ml usually sufficient) until the filtrate is free from chloride determined by silver nitrate test (NOTE 2).

Transfer the demineralized sample quantitatively to a 600-ml beaker with the aid of distilled water, and slurry the sample in 300 ml of distilled water. Heat sample dispersion in a steam bath or boiling water bath (NOTE 3), stirring continuously until the starch gelatinizes, and continue heating for 15 min to ensure complete gelatinization (NOTE 4).

Remove sample from bath and titrate while hot with standard 0.10 N sodium hydroxide solution to a phenolphthalein end-point. The end-point may be detected electrometrically at pH 8.3. A blank determination is run on the original sample to correct for native acid substances (Note 5). Weigh the same quantity of starch as taken for carboxyl titration, and slurry in 10 ml of distilled water. Stir at about 5-min intervals for 30 min.
Vacuum filter the slurry quantitatively through a medium porosity fritted-glass crucible or small funnel, and wash sample with 200 ml of distilled water. Transfer, gelatinize, and titrate the sample with standard 0.10 N sodium hydroxide in the same manner as the demineralized sample.

Calculation:

$$\text{Carboxyl groups (\%)} = \frac{(\text{ml 0.10N NaOH - Blank}) \times 0.0045 \times 100}{\text{Sample weight (g)}}$$

Notes and Precautions

1. Sample size should not exceed 5.0 g for a mildly oxidized or less than 0.15 g for a highly oxidized commercial starch.

2. Add 1 ml of 1% aqueous silver nitrate solution to 5 ml of filtrate. Turbidity or precipitation occurs within 1 min if chloride is present.

3. Heating on a hot plate or over a Bunsen burner is not recommended. Over-heating or scorching in amounts too small to be visible will cause sample decomposition and apparent high carboxyl results.

4. Thorough gelatinization facilitates rapid titration and accurate end-point detection.

5. A blank titration is run on a water-washed sample to correct for acidic components which are not introduced by oxidation or derivatization. Free fatty acids complexed with amylose in common corn starch are the principal contributors to the blank titre.

6. A correction for phosphate content in potato starch (deduction) should be made after determining the phosphorus content of the sample being examined.

The deduction is calculated:

$$\frac{2 \times 45.02 \times P}{30.97} = 2.907 \times P$$

where

P is the phosphorus content (%).

Phosphorus

Reagents

- Ammonium Molybdate Solution (5%): Dissolve 50 g of ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 900 ml of warm water, cool to room temperature, dilute to 1000 ml with water, and mix.

- Ammonium Vanadate Solution (0.25%): Dissolve 2.5 g of ammonium metavanadate, NH4VO3, in 600 ml of boiling water, cool to 60 - 70o, and add 20 ml of nitric acid. Cool to room temperature, dilute to 1000 ml with water, and mix.

- Zinc Acetate Solution (10%): Dissolve 120 g of zinc acetate dihydrate, Zn(C2H3O2)2·2H2O, in 880 ml of water, and filter through Whatman No. 2V or equivalent filter paper before use.

- Nitric Acid Solution (29%): Add 300 ml of nitric acid (sp. gr 1.42) to 600 ml of water, and mix.

- Standard Phosphorus Solution: (100 µg P in 1 ml): Dissolve 438.7 mg of monobasic potassium phosphate, KH2PO4, in water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Standard Curve

Pipet 5.0, 10.0, and 15.0 ml of the Standard Phosphorus Solution into separate 100-ml volumetric flasks. To each of these flasks, and to a fourth blank flask, add in the order stated 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg P per 100 ml.

Sample pre-treatment

Place 20 to 25 g of the starch sample in a 250-ml beaker, add 200 ml of a 7 to 3 methanol-water mixture, disperse the sample, and agitate mechanically for 15 min. Recover the starch by vacuum filtration in a 150 ml medium-porosity fritted-glass or Buchner funnel, and wash the wet cake with 200 ml of the methanol-water mixture. Reslurry the wet cake in the solvent, and wash it a second time in the same manner. Dry the filter cake in an air oven at a temperature below 50°, then grind the sample to 20-mesh or finer, and blend thoroughly. Determine the amount of dry substance by

drying a 5 g portion in a vacuum oven, not exceeding 100 mm of Hg, at 120° for 5 h. (NOTE: The treatment outlined above is satisfactory for starch products that are insoluble in cold water.

For pregelatinized starch and other water-soluble starches, prepare a 1% to 2% aqueous paste, place it in a cellophane tube, and dialyze against running distilled water for 30 to 40 h. Precipitate the starch by pouring the solution into 4 volumes of acetone per volume of paste, while stirring. Recover the starch by vacuum filtration in a medium-porosity fritted-glass or Buchner funnel, and wash the filter cake with absolute ethanol. Dry the filter cake, and determine the amount of dry substance as directed for water-insoluble starches).

Sample preparation

Transfer about 10 g of the Treated Sample, calculated on the dry-substance and accurately weighed, into a Vycor dish, and add 10 ml of Zinc Acetate Solution in a fine stream, distributing the solution uniformly in the sample. Carefully evaporate to dryness on a hot plate, then increase the heat, and carbonize the sample on the hot plate or over a gas flame. Ignite in a muffle furnace at 550° until the ash is free from carbon (about 1 to 2 h), and cool. Wet the ash with 15 ml of water and wash slowly down the sides of the dish with 5 ml of Nitric Acid Solution. Heat to boiling, cool, and quantitatively transfer the mixture into a 200-ml volumetric flask, rinsing the dish with three 20-ml portions of water and adding the rinsings to the flask. Dilute to volume with water, and mix. Transfer an accurately measured aliquot (V, in ml) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-ml volumetric flask and add 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min.

Procedure

Determine the absorbance of the Sample Preparation in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. From the Standard Curve, determine the mg of phosphorus in the aliquot taken, recording this value as a. Calculate the amount in mg/kg of Phosphorus (P) in the original sample by the formula:

$$\frac{a \times 200 \times 1000}{V \times W}$$

where

W is the weight of the sample taken, in g.

Manganese

Instrumentation
Atomic absorption spectrophotometer with manganese hollow cathode lamp.

Preparation of solutions
Standard solution: Prepare a solution containing 0.5 mg/l of manganese.
Sample solution: Transfer 10.000 g of the sample into a 200-ml Kohlrausch volumetric flask, previously rinsed with 0.5 N hydrochloric acid, add 140 ml of 0.5 N hydrochloric acid, and shake vigorously for 15 min, preferably with a mechanical shaker. Dilute to volume with 0.5 N hydrochloric acid, and shake. Centrifuge approximately 100 ml of the mixture in a heavy-walled centrifuge tube or bottle at 650xg for 5 min, and collect the supernatant liquid. This supernatant comprises the “sample solution”.

Procedure

Follow manufacturer's instructions for operating the atomic absorption spectrophotometer and aspirate distilled water through the air-acetylene burner for 5 min to obtain a base-line reading at 279.5 nm. In the same manner aspirate a portion of the "Standard solution" and note the reading. Finally, aspirate the "Sample solution" and compare the reading with the reading for the "Standard solution", and multiply this value by 20 to obtain mg per kg of manganese in the original sample taken for analysis.