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Hydroxypropyl Distarch Phosphate

(Tentative)

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HYDROXYPROPYL DISTARCH PHOSPHATE
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Hydroxypropyldistarch phosphate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:

- *A suitable method for the determination of propylene chlorohydrin*
- *A suitable test for identification of the phosphate groups*

SYNONYMS INS No. 1442

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose)and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
Hydroxypropyldistarch phosphate is a modified starch. It is obtained in accordance with good manufacturing practice by esterification of food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide.Hydroxypropylation results in substitution of hydroxyl groups with 2-hydroxypropyl ether. In cases of cross-linking, where phosphorus oxychloride, connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure.

Hydroxypropyldistarch phosphate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 53124-00-8
113894-92-1 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy	Passes test See description under TESTS
Iodine stain	Passes test See description under TESTS
Copper reduction	Passes test See description under TESTS
Phosphate groups	*Information required*
Hydroxypropyl ether groups	Passes test See description under TESTS

PURITY

Loss on drying	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Hydroxypropyl groups	Not more than 7.0%(calculated on dry substance) See description under TESTS
Propylene chlorohydrin	Not more than 1 mg/kg See description under TESTS
Phosphate (calculated as phosphorus) (Vol. 4)	Not more than 0.14% on the dried basis for potato and wheat starch Not more than 0.04% on the dried basis for other starches
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced
Hydroxypropyl ether groups[1]	Ninhydrin reagent A 3% solution of 1,2,3-triketohydrindene crystals in 4.55% aqueous sodium bisulfite solution. Procedure Weigh 100 mg of the sample into a 100-ml volumetric flask and add 12.5 ml of 2 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake). A violet colour develops only in the modified sample within 5 min due to the presence of hydroxypropyl groups (starch ether).For all other non-hydroxypropyl treated starches a light pink colour is observed.

PURITY TESTS

Phosphorus (Vol. 4)	Reagents – Ammonium Molybdate Solution (5%): Dissolve 50 g of ammonium molybdatetetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 900

[1]USP29-NF34: U.S. Pharmacopeial Convention, Hydroxylpropyl corn starch monograph, 2015. Reproduced with permission.

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, NH_4VO_3, 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, $Zn(C_2H_3O_2)_2 \cdot 2H_2O$, 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, KH_2PO_4, 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.

Hydroxypropyl groups

Ninhydrin reagent

A 3% solution of 1,2,3-triketohydrindene crystals in 5% aqueous sodium bisulfite solution.

Procedure

Accurately weigh 50 - 100 mg of the sample into a 100-ml volumetric flask and add 25 ml of 1 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. If necessary, dilute the sample further to assure the presence of no more than 4 mg of hydroxypropyl group per 100 ml, and then dilute the blank starch in the same proportion. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake). Immediately transfer portions of the solutions to 1-cm cells and after exactly 5 min, measure the absorption (A) at 590 nm, using the starch blank as the reference. Prepare a calibration curve with 1-ml aliquots of standard aqueous

solutions, containing 10, 20, 30, 40 and 50 µg of propylene glycol per ml.

Calculation

$$\text{Hydroxypropyl groups (\%)} = \frac{C \times 0.7763 \times 10 \times F}{W}$$

where

C is the amount of propylene glycol in the sample solution read from the calibration curve (µg/ml);
F is the dilution factor (if a further dilution has been necessary); and
W is the weight of sample (mg).

Propylene chlorohydrin *Information Required*