MODIFIED STARCHES

Prepared at the 79th JECFA and published in FAO JECFA Monographs 16 (2014), superseding the specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI “not specified” was established at the 26th JECFA (1982) for all modified starches listed below except for acetylated oxidized starch for which an ADI "not specified" was established at the 57th JECFA (2001).

Modified starches comprise the following:

Dextrin roasted starch: INS No. 1400
Acid treated starch: INS No. 1401
Alkaline treated starch: INS No. 1402
Bleached starch: INS No. 1403
Oxidized starch: INS No. 1404
Enzyme-treated starch: INS No. 1405
Monostarch phosphate: INS No. 1410
Distarch phosphate: INS No. 1412
Phosphated distarch phosphate: INS No. 1413
Acetylated distarch phosphate: INS No. 1414
Starch acetate: INS No. 1420
Acetylated distarch adipate: INS No. 1422
Hydroxypropyl starch: INS No. 1440
Hydroxypropyl distarch phosphate: INS No. 1442
Starch sodium octenylsuccinate: INS No. 1450
Acetylated oxidized starch: INS No. 1451

DEFINITION

Food starches which have one or more of their original characteristics altered by treatment in accordance with good manufacturing practice by one of the procedures listed in Table 1. In the case of starches treated with heat in the presence of acid or with alkali, the alteration is a minor fragmentation. When the starch is bleached, the change is essentially in the colour only. Oxidation involves the deliberate production of carboxyl groups. Acetylation results in substitution of hydroxyl groups with acetyl esters. Treatment with reagents such as orthophosphoric acid results in partial substitution in the 2, 3- or 6- position of the anhydroglucose unit unless the 6-position is occupied for branching. In cases of cross-linking, where a polyfunctional substituting agent, such as 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. The article of commerce can be specified by the parameter specific for the particular type of modification as indicated in Column 3 of Table 1, and may also be further specified as to the loss on drying, sulfated ash, protein and fat.

C.A.S. numbers

Starch acetate:	9045-28-7
Acetylated distarch adipate:	68130-14-3
Hydroxypropyl starch:	9049-76-7
Hydroxypropyl distarch phosphate:	53124-00-8
Acetylated oxidized starch:	68187-08-6
Starch sodium octenylsuccinate:	66829-29-6

DESCRIPTION

Most modified starches are white or off-white, odourless powders. According to the drying method these powders can consist of whole granules having the appearance of the original native starch, or aggregates consisting of a number of granules (pearl starch, starch-grits) or, if pre-gelatinized, of flakes, 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
Differentiation test	Passes test for type of starch See description under TESTS for: 1. Hypochlorite oxidized starch 2. Specific reaction for acetyl groups 3. Positive test for ester groups

PURITY

Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches Not more than 10 mg/kg for other modified starches unless otherwise specified in Table I See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS (electrothermal atomization technique) 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”).
Additional purity specifications for individual chemically modified starches	See column 3 of Table I See description under TESTS

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

Differentiation test

To differentiate between various treated starches perform the following tests:

1. Test for hypochlorite-oxidized starch (not for slightly oxidized potato starch)
Principle
Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue.

Procedure
50 mg of the sample are kept in suspension for 5-10 min in 25 ml of a 1% aqueous dye solution and stirred occasionally. After decantation of the excess solution, the starch is washed with distilled water. Microscopic inspection clearly shows colouring, if the sample is hypochlorite-oxidized starch. By this test hypochlorite-oxidized starch is distinguished from native and acid modified starch of the same botanical origin.

2. Specific reaction of acetyl groups
Principle
Acetate is liberated upon saponification of acetylated starch. After concentration the acetate is converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure
About 10 g of the sample is suspended in 25 ml water to which is added 20 ml of 0.4 N NaOH. After shaking for 1 h the starch is filtered off and the filtrate evaporated in an oven at 110°. The residue is dissolved in a few drops of water and transferred to a test tube. Add calcium hydroxide and heat the tube. If the sample is acetylated starch, acetone vapours are produced. These produce a blue colour on a paper strip soaked in a fresh saturated solution of o-nitrobenzaldehyde in 2 N NaOH. The blue colour is more distinct when the original yellow colour of the reagents is removed with 1 drop of a 1 in 10 solution of hydrochloric acid.

3. Positive test for ester groups
The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm-1 which is an indication for ester groups. The limit of detection is about 0.5% acetyl, adipyl or succinyl groups in the product.

PURITY TESTS

Sulfur dioxide

Scope

The method is applicable, with minor modifications, to liquid or solid samples even in the presence of other volatile sulfur compounds.

Principle

The sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in dilute hydrogen peroxide where it is oxidized to sulfuric acid and titrated with standard alkali. Alternatively, the sulfuric acid may be determined gravimetrically as barium sulfate.

Apparatus

“Monier-Williams" apparatus for the determination of sulfurous acid, constructed with standard-taper glass connections, can be obtained from any reliable scientific glass apparatus store. It is customary, however, to construct the apparatus with regular laboratory glassware using stopper connections (see Figure 1).

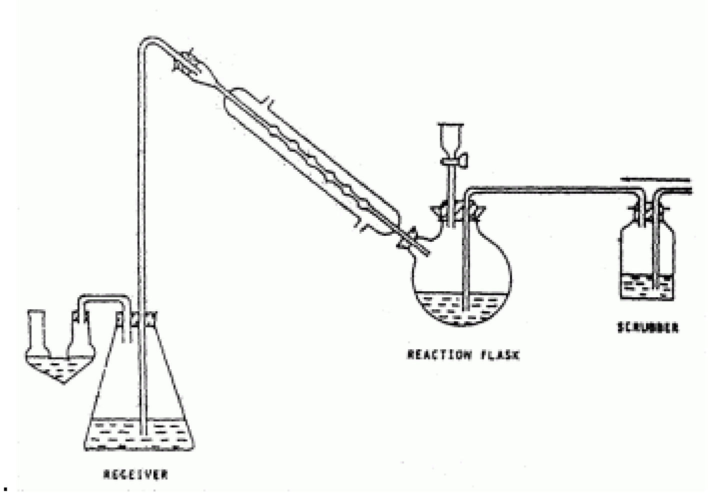

Figure 1

The assembly consists of a 1000-ml two-neck round-bottom boiling flask to which a gas-inlet tube, a 60-ml dropping funnel having a 2-mm bore stopcock, and a sloping Allihn reflux condenser are attached. A delivery tube connects the upper end of the condenser to the bottom of a 250-ml conical receiving flask, which is followed by a Peligot tube.

In operation, carbon dioxide is passed through the scrubber and bubbled through the heated reaction mixture, sweeping sulfur dioxide through the condenser and into the receivers where it is absorbed quantitatively.

Preparation of solutions

Sodium carbonate solution: Dissolve approximately 15 g of Na_2CO_3 or 40 g of $Na_2CO_3 \cdot 10H_2O$ in distilled water, and dilute to 100 ml.

Hydrogen peroxide, 3%: Dilute 10 ml of C.P. (Chemical Purity) neutral 30% hydrogen peroxide (H_2O_2) with distilled water to 100 ml.

Procedure

Pass carbon dioxide from a generator or cylinder through the sodium carbonate scrubber solution to remove chlorine, thence into the gas-inlet tube of the boiling flask. Place 15 ml of the 3% hydrogen peroxide in the receiving flask and 5 ml in the Peligot tube. Connect the apparatus and introduce into the boiling flask, by means of the dropping funnel, 300 ml of distilled water and 20 ml of concentrated hydrochloric acid. Boil the contents approximately 10 min in a current of carbon dioxide. Weigh, to the nearest g, 100 g of the sample and disperse in approximately 300 ml of recently-boiled distilled water. Transfer the slurry to the boiling flask by means of dropping funnel, regulating the sample addition rate and the gas flow rate through the apparatus to prevent drawback of hydrogen peroxide, inclusion of air, or burning of sample. Boil the mixture gently for 1 h in a slow current of carbon dioxide. Stop the flow of water in the condenser just before the end of the distillation. When the delivery tube just above the receiving flask becomes hot, remove the tube from the condenser immediately. Wash the delivery tube and the Peligot tube contents into the receiving flask, and titrate with 0.1 N sodium hydroxide, using bromphenol blue indicator (see NOTE).

Perform a blank determination on the reagents, and correct results accordingly.

$$\text{\% sulfur dioxide} = \frac{(S - B) \times 0.0032 \times 100}{W}$$

where

S is ml of 0.1 N sodium hydroxide used for the sample;
B is ml of 0.1 N sodium hydroxide used for the blank; and
W is the weight (in grams) of the sample.

NOTE: A gravimetric determination may be made after titration. Acidify with HCl, precipitate with $BaCl_2$, settle, filter, wash, ignite, and weigh as $BaSO_4$.

Table 1. Additional purity specifications for individual chemically modified starches
(All percentages calculated on dry substance)

Modification	Process limitations	End-product specifications
Dextrin roasted starch	Dry heat treatment with hydrochloric acid or ortho-phosphoric acid	Final pH 2.5-7.0
Acid treated starch	Treatment with hydrochloric acid or ortho-phosphoric acid or sulfuric acid	Final pH 4.8-7.0
Alkaline treated starch	Treatment with sodium hydroxide or potassium hydroxide	Final pH 5.0-7.5

Modification	Process limitations	End-product specifications
Bleached starch	Treatment with peracetic acid and/or hydrogen peroxide, or sodium hypochlorite or sodium chlorite, or sulfur dioxide or alternative permitted forms of sulfites, or potassium permanganate or ammonium persulfate	Added carbonyl group not more than 0.1% No residual reagent Residual sulfur dioxide not more than 50 mg/kg Residual manganese not more than 50 mg/kg
Enzyme-treated starch	Treatment in an aqueous solution at a temperature below the gelatinezation point with one or more food-grade amyolytic enzymes	Residual sulfur dioxide not more than 50 mg/kg
Oxidized starch	Treatment with sodium hypochlorite	Carboxyl groups not more than 1.1% Residual sulfur dioxide not more than 50 mg/kg
Monostarch phosphate	Esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate	Phosphate calculated as phosphorus not more than 0.5% for potato or wheat, and not more than 0.4% for other starches
Distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride	Phosphate calculated as phosphorus not more than 0.5% for potato and wheat, and not more than 0.4% for other starches
Phosphated distarch phosphate	Combination of treatments for Monostarch phosphate and Distarch phosphate	Phosphate calculated as phosphorus not more than 0.5% for potato and wheat, and not more than 0.4% for other starches
Acetylated distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate	Acetyl groups not more than 2.5%; phosphate calculated as phosphorus not more than 0.14% for potato and wheat, and 0.04% for other starches; and vinyl acetate not more than 0.1 mg/kg
Starch acetate	Esterification with acetic anhydride or vinyl acetate	Acetyl groups not more than 2.5%
Acetylated distarch adipate	Esterification with acetic anhydride and adipic anhydride	Acetyl groups not more than 2.5% and adipate groups not more than 0.135%
Hydroxypropyl starch	Etherification with propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg

Modification	Process limitations	End-product specifications
Hydroxypropyl distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg; and residual phosphate calculated as phosphorus not more than 0.14% for potato and wheat, and not more than 0.04% for other starches
Starch sodium octenylsuccinate	Esterification with octenylsuccinic anhydride	Octenylsuccinyl groups not more than 3%; and residual octenylsuccinic acid not more than 0.3%
Acetylated oxidized starch	Treatment with sodium hypochlorite followed by esterification with acetic anhydride	Acetyl groups not more than 2.5 % and carboxyl groups not more than 1.3 %

METHODS FOR ADDITIONAL PURITY SPECIFICATIONS

pH (Vol. 4)

As specified in Column 3 of Table 1
Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (In the case of pre-gelatinized starches, 3 g should be suspended in 97 ml of water).

Carboxyl groups

As specified in Column 3 of Table 1.

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:

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

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 (%).

Manganese (Vol. 4)

As specified in Column 3 of Table 1.

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.

Phosphorus (Vol. 4)

As specified in the Column 3 of Table 1.

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.

Acetyl groups

As specified in Column 3 of Table 1.

Accurately weigh about 5 g of the sample and transfer into a 250 ml conical flask. Suspend in 50 ml of water, add a few drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide to a permanent pink end-point. Add 25.0 ml of 0.45 N sodium hydroxide, stopper the flask, and shake vigorously for 30 min, preferably with a mechanical shaker. (NOTE: the temperature should not exceed 30° as some starches may gelatinize). Remove the stopper, wash the stopper and sides of the flask with a few ml of water, and titrate the excess alkali with 0.2 N hydrochloric acid to the disappearance of the pink colour. Record the volume, in ml of 0.2 N hydrochloric acid required as S.(S). Perform a blank titration on 25.0 ml of 0.45 N sodium hydroxide, and record the volume, in ml, of 0.2 N hydrochloric acid required as B.—(B).

$$\text{Acetyl groups (\%)} = \frac{(B - S) \times N \times 0.043 \times 100}{W}$$

where

N is the normality of hydrochloric acid solution; and
W is the weight of sample, In grams.

Vinyl acetate

- Headspace Gas Chromatographic method

Chromatographic system

Use a gas chromatograph equipped with a 2 m x 2 mm (i.d.) glass column containing Porapak Q, 80-100 mesh (or equivalent) fitted with a flame ionization detector, under the following conditions:

- Carrier gas flow (nitrogen): 20 ml/min
- injection port temperature: 200°
- column temperature: 50
- detector temperature: 200°

Standard preparation: Accurately weigh 150 mg vinyl acetate (reagent grade) into a 100 ml volumetric flask. Dissolve and make up to volume with distilled water. Place 1 ml of this solution in a 10-ml volumetric flask and make up to volume with distilled water. Add 1 ml of this dilute solution to 30 g unmodified starch of the same botanical origin as the test substance in a 100-ml flask with a septum-liner. Seal the flask immediately with the septum-liner. This provides a standard starch preparation with a vinyl acetate content of 5 mg/kg.

Procedure

Weigh 30 g of the test substance into a 100-ml flask with a septum-liner. Seal the flask. Place the flask containing the test substance and the flask containing the standard preparation in a constant temperature water bath at 70° for 30 min. Withdraw 2.0 ml from the headspace volume of the flask containing the standard preparation using a gas-tight syringe, inject directly into the injection port of the gas chromatograph and record the peak height of the chromatogram. Similarly inject 2.0 ml of the headspace volume from the flask containing the test substance into the chromatograph. Calculate the content of vinyl acetate in the test substance from a comparison of the peak heights of the two chromatograms.

Adipate groups

As specified in Column 3 of Table 1.

Reagents and Solutions

N,N-Bis-trimethylsilyltrifluoroacetamide (BSTFA): Macherey-Nagel, D 5160 Dueren, Germany or equivalent.
Glutaric acid solution: Dissolve 1.00 g of glutaric acid (Merck or equivalent) in water and dilute to 1000 ml.

Adipic acid solution: Dissolve 1.00 g of adipic acid (UCB, Brussels, Belgium or equivalent) in 900 ml of warm water, cool to room temperature, dilute to 1000 ml and mix.

Apparatus

Chromatograph: Hewlett Packard Model 7620A gas chromatograph or equivalent equipped with flame ionization detector and Model 3370Aintegrator. (Hewlett-Packard Model 7620A, with integrator Model 3370A or equivalent)

Column parameters: 2-m stainless steel, 1.83 mm id, packed with 5% OV-17 on 80-100 mesh Chromosorb GAW-DMCS (Alltech Europe, Inc., B 9731 Eke, Belgium); precondition column 24 h at 350° with nitrogen carrier gas at 40 ml/min. Operating gas flow rates (ml/min): nitrogen carrier 30, hydrogen 40, air 400. Temperature: injection 280°, detector 250°, column 140°. Retention times (min): glutaric acid 2.83, adipic acid 4.50.

Calibration

Weigh 1.0 g waxy corn starch into each of four 250-ml Erlenmeyer flasks. To each flask add 50 ml water and 1.0 ml of an aqueous solution containing 1.0 mg glutaric acid/ml. Add, to one flask, 0.25 ml of an aqueous solution containing 1.0 mg adipic acid per ml; to the other three, add 0.50 ml, 0.75 ml, and 1.0 ml, respectively. Each flask then contains 1.0 mg glutaric acid and, respectively, 0.25, 0.50, 0.75 and 1.0 mg adipic acid. Agitate flasks manually to disperse the starch fully and add 50 ml 4N sodium hydroxide. Continue agitation another 5 min, place each flask in water bath at ambient temperature, and carefully add 20 ml 12 N hydrochloric acid to each. When each flask is cool quantitatively transfer contents to 250 ml separatory funnel. Extract with 100 ml reagent grade ethyl acetate. Drain bottom aqueous layer into beaker and collect upper organic layer in 500-ml Erlenmeyer flask containing 20 g anhydrous sodium sulphate. Transfer aqueous portion back to separatory funnel and repeat ethyl acetate extraction twice more. Shake flasks periodically during 10 min and then filter contents through Whatman No. 1 paper into 1-litre round-bottom flasks. Rinse flasks and insoluble residues in filters twice with 50 ml of ethyl acetate. Under vacuum, (50 mm Hg) at temperature not exceeding 40°, evaporate total organic extraction and washings of each flask until completely dry.

The evaporation of ethyl acetate should be effected as quickly as possible because some hydrolysis takes place on standing. The products of hydrolysis cause deterioration in the resolution of adipic acid in the chromatographic separation.

Successively add 2 ml pyridine and 1 ml N,N-bis-trimethylsilyltrifluoro-acetamide to the dry contents. Close each of the round-bottom flasks with stopper and rinse internal surfaces thoroughly by swirling. Let flasks stand 1 h; then transfer ca 2 ml from each to small glass vials and immediately seal. Inject 4 µl into gas chromatograph.

Calculations

Establish retention times for each acid and determine peak height for glutaric acid and for each level of adipic acid represented. A plot of peak height ratio of adipic acid to glutaric acid against amount of adipic acid is linear. This calibration curve may be used, but it is simpler to use a response factor (RF):

$$RF = \frac{H_I \times W_S}{H_S}$$

where

H_S and H_I is the peak heights of the standard adipic acid and glutaric acid, respectively; and

W_S is the weight of the standard adipic acid.

RF should be verified weekly.

Total adipate

Accurately weigh about 1.0 g of the sample into a 250 ml Erlenmeyer flask, and add 50 ml water and 1.0 ml of an aqueous solution containing 1.0 mg glutaric acid/ml. Proceed as in Calibration, beginning "Agitate flasks manually...".

Free adipic acid

Accurately weigh about 5.0 g of the sample into a 250 ml Erlenmeyer flask, add 100 ml water and 1.0 ml of the glutaric acid solution. Agitate for 1 h, filter through a 0.45 µm Millipore filter, add 1 ml concentrated hydrochloric acid to the filtrate and transfer it quantitatively to a 250-ml separating funnel. Proceed as in Calibration, beginning "Extract with 100 ml..."

Calculation

For both preparations ("Total adipate content" and "Free adipic acid content") record peak heights for adipic acid and glutaric acid (internal standard). Calculate the amounts of total adipate and free adipic acid, respectively, contained in the sample as follows:

$$A = \frac{H_X \times RF}{H_{IX} \times S \times 10}$$

where

A is the content of total adipate or free adipic acid respectively (%);

H_X is the peak height of adipic acid in the actual sample preparation;

H_{IX} is the peak height of glutaric acid in the actual sample preparation;

RF is the response factor for adipic acid; and

S is the weight of sample in the actual preparation (g).

Adipate groups (%) is equal to content of total adipate (%) - content of free adipic acid (%).

Hydroxypropyl groups

As specified in Column 3 of Table 1

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

As specified in Column 3 of Table 1.
Determine by gas chromatography

Gas Chromatographic system
Use a Hewlett Packard model 5750 or equivalent.. A dual-column and a flame-ionization detector is recommended.. An integrator should be part of the recording system

Gas Chromatography column: Use a stainless steel column, 3 m x 3.2 mm (o.d.), packed with 10% Carbowax 20 M on 80/100-mesh Gas Chrom 2, or equivalent. After packing and prior to use, condition the column overnight at 200°, using a helium flow of 25 ml per min.
Concentrator: Use a Kuderna-Danish concentrator having a 500-ml flask, available from Kontes Glass Co., Vineland, N.J., USA, (Catalogue No. K-57000), or equivalent.

Pressure Bottles: Use 200-ml pressure bottles, with a Neoprene washer, glass stopper, and attached wire clamp, available from Fisher Scientific Co., Pittsburgh, PA, USA (Vitro 400, Catalogue No. 3-100), or equivalent.

Reagents
Diethyl ether: Use anhydrous, analytical reagent-grade, diethyl ether.
Florisil: Use60/100 mesh material,, available from Floridin Co., 3 Penn Center, Pittsburgh, PA 15235, USA, or an equivalent product.
Propylene chlorohydrins: Use Eastman No. P 13251-Chloro-2-propanol Practial containing 25% 2-chloro-1-propanol available from Eastman Kodak Co., Rochester, N.Y. 14650, USA or equivalent).

Standard preparation

Draw 25 µl of mixed propylene chlorohydrin isomers containing 75% of 1-chloro-2-propanol and 25% of 2-chloro-propanol into a 50-µl syringe. Accurately weigh the syringe and discharge the contents into a 500-ml volumetric flask partially filled with water. Reweigh the syringe, and record the weight of the chlorohydrins taken. Dilute to the volume with water, and mix. This solution contains about 27.5 mg of mixed chlorohydrins, or about 55 µg per ml. Prepare this solution fresh on the day of use.

Sample preparation

Transfer a blended representative 50.0 g sample into a Pressure Bottle, and add 125 ml of 2 N sulfuric acid. Clamp the top in place, and swirl the contents until the sample is completely dispersed. Place the bottle in a boiling water bath, heat for 10 min, then swirl the bottle to mix the contents, and heat in the bath for an additional 15 min. Cool in air to room temperature, then neutralize the hydrolyzed sample to pH 7 with 25% sodium hydroxide solution, and filter through Whatman No. 1 paper, or equivalent, in a Buchner funnel, using suction. Wash the bottle and filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, and stir with a magnetic stirring bar for 5 to 10 min, or until the sodium sulfate is completely dissolved. Transfer the solution into a 500-ml separator equipped with a teflon plug, rinse the flask with 25 ml of water, and combine the washings with the sample solution. Extract with five 50 ml portions of diethyl ether, allowing at least 5 min in each extraction for adequate phase separation. Transfer the combined ether extracts in a Concentrator, place the graduated receiver of the concentrator in a water bath maintained at 50 - 55°, and concentrate the extract to a volume of 4 ml.

(NOTE: Ether extracts of samples may contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. These residues are believed to be degradation products arising during the hydrolysis treatment. Analytical problems created by their presence can be avoided through application of a clean-up treatment performed as follows: Concentrate the ether extract to about 8 ml, instead of 4 ml specified above. Add 10 g of Florisil, previously heated to 130° for 16 h just before use, to a chromatographic tube of suitable size, then tap gently, and add 1 g of anhydrous sodium sulfate to the top of the column. Wet the column with 25 ml of diethyl ether, and quantitatively transfer the concentrated extract to the column with the aid of small portions of the ether. Elute with three 25-ml portions of the ether, collect all of the eluate, transfer it to a concentrator, and concentrate to a volume of 4 ml).

Cool the extract to room temperature; transfer it quantitatively to a 5.0 ml volumetric flask with the aid of small portions of diethyl ether, dilute to volume with the ether, and mix.

Control preparation

Transfer 50.0 g portions of unmodified (underivatized) waxy corn starch into five separate pressure bottles, and add 125 ml of 2 N sulfuric acid to each bottle. Add 0.0, 0.5, 1.0, 2.0, and 5.0 ml of the Standard Preparation to the bottles, respectively, giving propylene chlorohydrin concentrations, on the starch basis, of 0, 0.5, 1.0, 2.0, and 5.0 mg/kg, respectively. Calculate the exact concentration in each bottle from the weight of propylene chlorohydrins used in making the Standard Preparation. Clamp the tops in place, swirl until the contents of each bottle are completely dissolved, and proceed with the hydrolysis, neutralization, filtration, extraction, extract concentration, and final dilution as directed under Sample Preparation.

Procedure

The operating conditions may be varied, depending upon the particular instrument used, but a suitable chromatogram is obtained with the Hewlett-Packard Model 5750 using a column oven temperature of 110°, isothermal; injection port temperature of 210°; detector temperature of 240°; and hydrogen (30 ml per min), helium (25 ml per min), or air (350 ml per min) as the carrier gas. A 1.0 mV full-scale recorder is recommended; range, attenuation, and chart speed should be selected to optimize signal characteristics. Inject 2.0 µl aliquots of each of the concentrated extracts, prepared as directed under Control preparation, allowing sufficient time between injections for signal peaks corresponding to the two chlorohydrin isomers to be recorded (and integrated) and for the column to be purged. Record and sum the signal areas (integrator outputs) from the two chlorohydrin isomers for each of the controls. Using identical operating conditions, inject a 2.0 µl aliquot of the concentrated extract prepared as directed under Sample preparation, and record and sum the signal areas (integrator outputs) from the sample.

Calculation

Prepare a calibration plot on linear coordinate graph paper by plotting the summed signal areas for each of the controls against the calculated propylene chlorohydrin concentrations, in mg/kg, derived from the actual weight of chlorohydrin isomers used. Using the summed signal areas corresponding to the 1-chloro-2-propanol and 2-chloro-1-propanol from the sample, determine the concentration of mixed propylene chlorohydrins, in mg/kg, in the sample by reference to the calibration plot derived from the control samples. After gaining experience with the procedure and demonstrating that the calibration plot derived from the control samples is linear and reproducible, the number of controls can be reduced to one containing about 5 mg/kg of mixed propylene chlorohydrin isomers. The propylene chlorohydrin level in the sample can then be calculated as follows:

$$\text{Propylene chlorohydrins (mg/kg)} = \frac{C \times a}{A}$$

where

C is the concentration, in mg/kg, of propylene chlorohydrins (sum of isomers) in the control;
a is the sum of signal areas produced by the propylene chlorohydrin isomers in the sample; and
A is the sum of the signal areas produced by the propylene chlorohydrin isomers in the control.

Octenylsuccinyl groups in starch sodium octenyl succinate

Principle

The sample is equilibrated with mineral acid to convert octenyl succinate salts to the acid form. Cations and excess acid are removed by thorough washing with 90% isopropanol in water. The washed sample is titrated with standard alkali.

Procedure

Weigh accurately about 5.000 g of sample into a 150-ml beaker and wet the sample with a few ml of isopropanol. Add 25 ml of 2.5 M hydrochloric acid in isopropanol, allowing the acid to wash down any sample on the sides of the beaker. Stir the mixture with a magnetic stirrer for 30 min. Using a graduated measuring cylinder, add 100 ml of 90% isopropanol in water and stir the contents for another 10 min. Filter through a Buchner funnel and wash the filter cake with 90% isopropanol in water until the filtrate is negative for chloride (check using 0.1 N silver nitrate). Quantitatively transfer the filter cake into a 600-ml beaker using distilled water and, making sure to rinse the Buchner funnel to wash any starch into the beaker. Bring to about 300-ml using distilled water. Place the beaker on a boiling water bath for 10 min with stirring. Titrate, while hot, with 0.1 N sodium hydroxide using phenolphthalein TS as an indicator. Repeat the titration procedure with native unmodified starch of the same origin as the OSA starch sample, as a blank.

Calculation

$$\text{Octenyl succinyl groups (\%)} = \frac{21.1 \text{ x } N \text{ x } [V_{sample} - V_{blank}]}{W}$$

where

V_{sample} is the titration volume of sodium hydroxide for the sample, ml
V_{blank} is the titration volume of sodium hydroxide for the blank, ml
N is the normality of sodium hydroxide
W is the dry weight of sample, g

Residual octenyl succinic acid in starch sodium octenyl succinate

Determine by HPLC on the 2-bromoacetophenone-derivatised methanolic extract of the sample.

Extraction and Preparation of Sample Solution

Accurately weigh 500 mg (to nearest 0.1 mg) of the sample in a 25 ml Erlenmeyer flask, add 15 ml of methanol, stopper the flask and shake it on a shaker overnight. Filter the extract using a filter paper, wash the residue, three times with 7 ml portions of methanol and combine the filtrate (about 80% of the OSA residues is extracted by this procedure). Add 1 ml of 0.16 N KOH in methanol to the combined filtrate. Dry the extract using a flash evaporator at 30° and dissolve the residue in 2 ml of methanol. Pipette 0.5 ml of this solution into a reaction vial, add 0.5 ml of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml CH_3CN]. Add 2 ml CH_3CN to the reaction vial, cap the vial and heat at 80° for 30 min. Allow the vial to reach room temperature and analyse by HPLC within 24 h.

HPLC Conditions:

Column: µ-Bondapack C18 or equivalent
Mobile Phase: Methanol and Water with gradient elution: 70% to 80% of methanol in water in 5 min
Flow rate: 1.5 ml/min
Detector: UV at 254 nm
Injection volume: 5 µl

Preparation of Standard Curve

Prepare a 105.14 mg/ml solution of octenyl succinic acid anhydride (available from Milliken Chemicals) in methanol (Solution A). Using a syringe draw 0.25 ml of Solution A, transfer into a 25-ml volumetric flask and dilute to mark with methanol (Solution B).

Prepare three working standards (Solution C1, C2 and C3) by transferring 0.5, 1 and 2 ml each of Solution B into three 50-ml round bottom flasks, add 1 ml of 0.16 N KOH in methanol to each flask, dry the solution using a flash evaporator at 30° and dissolve the residue in 2.0 ml of methanol.

To 0.5 ml each of these solutions in reaction vials, add 0.5 ml each of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml of CH_3CN]. Add 2 ml of CH_3CN to each vial; cap the vials and heat for 30 min at 80°. Allow the vials to reach room temperature and analyze by HPLC immediately. The amount of octenyl succinic acid in each 5-µl injection is as follows:

Solution C1: 0.2375 µg
Solution C2: 0.4750 µg
Solution C3: 0.9500 µg

Construct the standard curve using peak height against the amount of standard in the injection.

Inject 5-µl of prepared sample solution and read the amount of octenyl succinic acid in the injection from the standard curve.

Calculation

$$\text{\% Residual octenyl succinic acid} = \frac{300 \times V}{W}$$

where

V is the amount of OSA in the injected volume; and
W is the weight of the sample (mg).

NOTE: The formula is corrected to 100% recovery by dividing with 0.80, so that 240/0.80 = 300.