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MODIFIED STARCHES

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MODIFIED STARCHES

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications included in 16 individual specification monographs prepared at the 82nd JECFA (2016), published in FAO JECFA Monographs 19 (2016).

MODULAR MONOGRAPH consisting of "GENERAL SPECIFICATIONS"[(a)] that contains common specifications to all modified starches(INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and 8 ANNEXES that contain specifications related to the chemical treatments of native starches:

- ANNEX 1[(a)] – Fragmentation.
- ANNEX 2[(a)] – Bleaching.
- ANNEX 3[(a)] – Esterification and/or crosslinking with phosphorous containing compounds.
- ANNEX 4[(a)] – Acetylation.
- ANNEX 5[(a)] – Oxidation.
- ANNEX 6[(a)] – Esterification with octenyl succinic anhydride.
- ANNEX 7[(a)] – Etherification with propylene epoxide.
- ANNEX 8[(a)] – Esterification and crosslinking with adipic anhydride.

The General specifications are applicable to the following modified starches, each of which should additionally fulfil the specifications of the ANNEXES as follows:

Modified Starch	INS	Annex	ADI	STATUS
Dextrin roasted starch	1400	1	N.S.[(1)]	T[(3)]
Acid treated starch	1401	1	N.S.[(1)]	T[(3)]
Alkaline treated starch	1402	1	N.S.[(1)]	T[(3)]
Bleached starch	1403	2	N.S.[(1)]	T[(3)]
Oxidized starch	1404	5	N.S.[(1)]	T[(3)]
Enzyme-treated starch	1405	1	N.S.[(1)]	T[(3)]
Monostarch phosphate	1410	3	N.S.[(1)]	T[(3)]
Distarch phosphate	1412	3	N.S.[(1)]	T[(3)]
Phosphated distarch phosphate	1413	3	N.S.[(1)]	T[(3)]

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Acetylated distarch phosphate	1414	3, 4	N.S.[1]	T[3]
Starch acetate	1420	4	N.S.[1]	T[3]
Acetylated distarch adipate	1422	4, 8	N.S.[1]	T[3]
Hydroxypropyl starch	1440	7	N.S.[1]	T[3]
Hydroxypropyl distarch phosphate	1442	3, 7	N.S.[1]	T[3]
Starch sodium octenylsuccinate	1450	6	N.S.[1]	T[3]
Acetylated oxidized starch	1451	4, 5	N.S.[2]	T[3]

Should any of the modified starches be subjected to additional chemical treatment, the appropriate specifications outlined in the respective ANNEX should be met. Consequently, for all fragmented and/or bleached starches the specifications of ANNEXES 1 and/or 2 respectively should be met.

[a]Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications included in specification monographs prepared at the 82nd JECFA (2016), published in FAO JECFA Monographs 19(2016).

[1]An ADI "not specified" was established at the 26th JECFA (1982).
[2]An ADI "not specified" was established at the 57th JECFA (2001).
[3] T: TENTATIVE

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Summary Table								
	GENERAL REQUIREMENTS							
		IDENTIFICATION		PURITY				
Solubility	**Microscopy**	**Iodine Stain**	**Copper Reduction**	**pH**	**Loss on Drying**	**Lead**	**Microbiological Criteria**	**Sulfur dioxide**
Insoluble in cold water	Granular structure typical of the starch source	Colour from dark blue to red after addition of tri-iodide	Red precipitate after addition of hot alkaline cupric tartrate to a test sample refluxed under acidic condition	**3.0 -9.0**	Cereal starch ≤15.0%; Potato starch: ≤21.0%; Other starches: ≤18.0%	≤0.2mg/kg d.w. Pb (≤0.1 mg/kg) for OSA for infant formula	Aerobic Plate Count: ≤1000 CFU/g; Yeasts and molds: ≤1000 CFU/g; Total Coliforms: ≤10 cfu/g; Information required.	≤50 mg/kg d.w. for modified cereal starches; ≤10 mg/kg d.w. for other modified starches

	SPECIFIC REQUIREMENTS		
Modified Starch	Annex	**IDENTIFICATION**	**PURITY**
Dextrin roasted (INS 1400)	1	Dispersion index (Information Required); Reducing sugars (Information Required)	No additional
Acid treated (INS 1401)	1	Dispersion index (Information Required); Reducing sugars (Information Required)	No additional
Alkaline treated starch (INS 1402)	1	Dispersion index (Information Required); Reducing sugars (Information Required)	No additional
Bleached (INS 1403)	2	No additional	Carboxyl groups (≤0.1% d.w.);Residual oxidising substances (Information Required)
Oxidized (INS 1404)	5	hypochlorite oxidized starch	Carboxyl groups (≤1.3% d.w.);Residual hypochlorite (Information Required)
Enzyme-treated (INS1405)	1	Dispersion index (Information Required); Reducing sugars (Information Required)	No additional
Monostarch phosphate (INS 1410)	3	Information required	Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.wfor other starches)
Distarch phosphate (INS 1412)	3	Information required	Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.w. for other starches)
Phosphated distarch phosphate (INS 1413)	3	Information required	Phosphate (≤0.5% d.w. for potato or wheat starches; ≤0.4% d.w. for other starches)
Acetylated distarch phosphate (INS 1414)	3, 4	Acetyl group; Ester group; Information required	Phosphate (≤0.14% d.w. for potato or wheat starches; ≤0.04% d.w. for other starches) Acetyl groups (≤2.5%d.w.); Ester groups (≤0.5%d.w.)
Starch acetate (INS 1420)	4	Acetyl group; Ester group	Acetyl groups (≤2.5%d.w.); Ester groups (≤0.5%d.w.)
Acetylated distarch adipate (INS 1422)	4, 8	Acetyl group; Ester group; Information required	Acetyl groups (≤2.5%d.w.); Vinyl acetate (≤0.1 mg/kg); Ester groups (≤0.5%d.w.) Adipate groups (≤0.135%d.w.); Residual free adipic acid (Information Required)
Hydroxypropyl starch (INS 1440)	7	Hydroxypropyl ether groups	Hydroxypropyl groups (≤7.0% d.w.); Propylene chlorohydrins (≤1 mg/kg d.w.)
Hydroxypropyl distarch phosphate (INS 1442)	3, 7	Hydroxypropyl ether groups; Information required	Phosphate (≤0.14% d.w. for potato or wheat starches; ≤0.04% d.w. for other starches) Hydroxypropyl groups (≤7.0% d.w.); Propylene chlorohydrins (≤1 mg/kg d.w.)
Starch sodium octenylsuccinate (INS 1450)	6	No additional	Octenylsuccinyl groups (≤3% d.w.); Residual free octenylsuccinic acid (≤0.3%d.w.);
Acetylated oxidized starch (INS 1451)	4, 5	Acetyl group	Acetyl groups (≤2.5%d.w.); Vinyl acetate (≤0.1 mg/kg); Ester groups (≤0.5%d.w.) Carboxyl groups (≤1.3% d.w.);Residual hypochlorite (Information Required)

GENERAL SPECIFICATIONS FOR MODIFIED STAI

(VERSION 2018 - TENTATI

Information required:

- *Suitable microbiologi( )*
  data

DEFINITION

Starch consists mainly of amylose and amylopectin. Amylose is a linear molecule of α-D-glucopyranosyl units linked by (1-4)-α-linkages. Amylopectin is a highly-branched polymer of α-D-glucopyranosyl units linked by (1-4)-α-linkages and by(1-6)-α-linkages that constitute the branch points. Each glucose unit possesses a maximum of three hydroxyls that can undergo chemical substitution.

Native starches can be physically (pre-gelatinized starches) and/or chemically modified for improved functionality. The most common sources of native starch used in these modifications are various roots, tubers, cereals and legumes. Modified starches are used in applications requiring special properties not attainable by native starches.

Chemical modifications of native starches are often performed, in an aqueous suspension under controlled conditions of pH, time and temperature, unless otherwise indicated in the description of the respective annex. After sufficient reaction time, the modified starch is recovered by filtration or centrifugation, washed with water, dried and packaged. The relevant modification reactions can be, separately or in combination, fragmentations (hydrolysis, oxidation, enzymatic), bleaching, oxidation, esterification, etherification or phosphorylation of one or more of the hydroxyl groups of the α-D-glucopyranosyl units or crosslinking using polyfunctional agents.

See the appropriate Annex or Annexes for the treatment that is applicable to individual modified starch products.

C.A.S numbers

See ANNEXES

DESCRIPTION

White or nearly white powder or granules or (if pre-gelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES

Thickener, stabilizer, binder, emulsifier

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CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in cold water (if not pre-gelatinised); 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

PURITY

General Requirements:

pH	3.0 – 9.0 See description under TESTS
Loss on drying (Vol 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% Conditions: 120°, 4 h, vacuum not exceeding 100 mm Hg
Lead (Vol. 4)	Not more than 0.2 mg/kg on the dried basis Not more than 0.1 mg/kg on the dried basis for Starch sodium octenylsuccinate (INS 1450) for use in infant formula and formula for special medical purposes intended for infants(see Annex 6)

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	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").
Microbiological Criteria (Vol 4)	Aerobic plate count: Not more than 1000 CFU/g Yeasts and moulds: Not more than 1000 CFU/g Total coliforms: Not more than 10 CFU/g Information required
Sulphur 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

TESTS

IDENTIFICATION TESTS

Microscopy	Each modified starch, which has not been pre-gelatinized, retains its granular structure and can be identified as a starch by microscopic observation. The typical polarization cross is observed when sample is examined with a polarizing microscope, in polarized light under crossed Nicol prisms. Corn starch: Polygonal, rounded or spherical granules up to 35 µm diameter having a circular or several-rayed central cleft. Potato starch: Irregular shaped, ovoid, pear-shaped granules (30-100 µm diameter, occasionally >100 µm); both, the ovoid, the pear-shaped granules and the rounded granules have an eccentric hilum. All granules show clearly visible concentric striations. Tapioca starch: Spherical granules with one truncated side (5-35 µm diameter) usually having a circular or several-rayed central cleft. Wheat starch: large and small granules (10-60 µm diameter). The central hilum and striations are visible and barely visible.
Iodine stain	Add a few drops of 0.1 N potassium triiodide to an aqueous suspension of the sample. The modified starch stains with iodine in the same way as native starches. The colour can range from dark blue to red.

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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.

PURITY TESTS

pH (Vol. 4) — 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-gelatinised starches, 3 g should be suspended in 97 ml of water).

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ANNEX 1: ADDITIONAL SPECIFICATIONS FOR STARCHES MODIFIED BY FRAGMENTATION

(VERSION 2018 - TENTATIVE)

Information is required on:

- *A suitable method for dispersion and a method for reducing sugars and data on at least 5 representative batches using the method(s) from each of the fragmentation processes*

APPLIES TO

Dextrin roasted starch (INS No. 1400)

Acid treated starch (INS No. 1401)

Alkaline treated starch (INS No. 1402)

Enzyme-treated starch (INS No. 1405)

All modified starches that are fragmented

SYNONYMS

Modified starch by fragmentation, converted starch, hydrolysed starch.

TREATMENT

The fragmentation of native starch results in products containing polymers with a lower average molecular weight and reduced viscosity. The manufacturing details for the various modified starches by fragmentation in this monograph are described as below:

- *Dextrin roasted starch, INS. 1400*: is manufactured by dry heating or roasting of native starch with hydrochloric acid or ortho-phosphoric acid in heated and/or agitated vessels. The final dextrin roasted starch is obtained by drying.
- *Acid treated starch, INS. 1401* is obtained by treating a slurry or a suspension of native food starch with dilute hydrochloric acid, ortho-phosphoric acid, or sulphuric acid.
- *Alkaline treated starch, INS. 1402* is obtained by treating a suspended solution of native food starches with sodium hydroxide or potassium hydroxide.
- *Enzyme-treated starch, INS 1405* is obtained by treating a suspension of native food starch with one or more food-grade amyolytic-enzymes (e.g., α-amylase (E.C. 3.2.1.1), β-amylase (3.2.1.2), glucoamylase (3.2.1.3), isoamylase (3.2.1.68), pullulanase (E.C. 3.2.1.41)).

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The properties of the modified starches by fragmentation vary depending on the source of native starch, reaction conditions (pH, reaction time, reaction temperature, fragmenting reagent etc.) The alteration of native starch allows for applications that require reduced viscosity in hot solutions and/or typically utilise high levels of modified starches.

C.A.S number

9004-53-9 (Dextrins)

65996-63-6 (Acid-hydrolysed starch)

68909-37-5 (Acid-hydrolysed amylopectin)

9005-84-9 (Starch soluble)

65996-64-7 (Enzyme-hydrolysed starch)

1001439-91-3 (Enzyme-treated amylopectin).

CHARACTERISTICS

IDENTITY

Dispersion identity — Information required.

Reducing sugars — Information required

TESTS

IDENTIFICATION TESTS

Dispersion test — Information required

Reducing sugars — Information required

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ANNEX 2: ADDITIONAL SPECIFICATIONS FOR BLEACHED STARCHES

(VERSION 2018 - TENTATIVE)

Information is required on:

- *Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s).*

APPLIES TO	Bleached starch INS No. 1403 All modified starches that are bleached
TREATMENT	Peracetic acid and/or hydrogen peroxide, or sodium hypochlorite, sodium chlorite, sulfur dioxide, alternative permitted forms of sulphites, potassium permanganate, or ammonium persulfate Bleaching is performed to improve physical attributes such as colour due to oxidation of traces of pigments such as carotenoids and xanthophylls. The change is essentially in the colour only. Residual reagents are either removed or limited to technically unavoidable levels.
C.A.S number	977075-42-5 and all other modified starches submitted to bleaching

CHARACTERISTICS

PURITY

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").
Residual oxidising substances	Information required
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis applying the correction for phosphate content as outlined in Note 6 of the method for starches esterified with phosphorus containing compounds.

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ANNEX 3: ADDITIONAL SPECIFICATIONS FOR STARCHES ESTERIFIED AND/OR CROSSLINKED WITH PHOSPHORUS CONTAINING COMPOUNDS

(VERSION 2018 - TENTATIVE)

Information required on: A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches.

APPLIES TO

Monostarch phosphate (INS No. 1410)

Distarch phosphate (INS No. 1412)

Phosphated distarch phosphate (INS No. 1413)

Acetylated distarch phosphate (INS No. 1414)

Hydroxypropyl distarch phosphate (INS No. 1442)

TREATMENT

The phosphorus containing compounds ortho-phosphoric acid, sodium or potassium ortho-phosphate and sodium tripolyphosphate, can be used for esterification and the sodium trimetaphosphate or phosphorus oxychloride for crosslinking.

- *Monostarch phosphate (INS 1410)* is obtained by esterification/crosslinking of unmodified food starch with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate
- *Distarch phosphate (INS 1412)* is obtained by crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride
- *Phosphated distarch phosphate (INS 1413)* is obtained by esterification/crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate
- *Acetylated distarch phosphate (NS 1414)* is obtained by esterification/crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate
- *Hydroxypropyl distarch phosphate (INS1442)* is obtained by esterification of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide

Phosphorylation results in partial substitution of the 2, 3- or 6-position of the anhydro glucose unit unless the 6-position is occupied for branching. In the case of cross-linking, where a polyfunctional substituting agent, such as phosphorus

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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.

C.A.S numbers

Monostarch phosphate (INS 1410)
11120-02-8(Modified starch)
63055-37-8 (Modified amylopectin)

Distarch phosphate (INS No. 1412)
55963-33-2(Modified starch)
63055-37-8 (Modified amylopectin)

Phosphated distarch phosphate (INS No. 1413)
11120-02-8(Modified starch)
63055-37-8 (Modified amylopectin)

Acetylated distarch phosphate (INS No. 1414)
9067-33-8(Modified starch)
68130-14-3(Modified starch)
113894-91-0 (Modified amylopectin)

Hydroxypropyl distarch phosphate (INS No. 1442)
53124-00-8(Modified starch)
113894-92-1 (Modified amylopectin)

CHARACTERISTICS

PURITY

Phosphate (calculated as phosphorus) (Vol. 4)

For monostarch phosphate (INS No. 1410), distarch phosphate (INS No. 1412), and phosphate distarch phosphate (INS No. 1413)

Not more than 0.5% on the dried basis for potato or wheat starches

Not more than 0.4% on the dried basis for other starches

For acetylated distarch phosphate (INS No. 1414) and hydroxypropyl distarch phosphate (INS No. 1442)

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

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IDENTITY

Crosslinking Information Required

ANNEX 4: ADDITIONAL SPECIFICATIONS FOR ACETYLATED STARCHES

Version 2018

APPLIES TO

Acetylated distarch phosphate (INS No. 1414)

Starch acetate (INS No. 1420)

Acetylated distarch adipate (INS No. 1422)

Acetylated oxidized starch (INS No. 1451)

TREATMENT

This type of modified starch is obtained by esterification with acetic anhydride or vinyl acetate. Acetylation results in substitution of hydroxyl groups with acetyl esters.

- *Acetylated distarch phosphate (INS 1414)* is obtained by esterification/cross-linking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate.
- *Starch acetate (INS 1420)* is obtained by esterification of food starches with acetic anhydride or vinyl acetate
- *Acetylated distarch adipate (INS 1422)* is obtained by esterification of unmodified food starch with acetic anhydride and esterification/cross-linking with adipic anhydride
- *Acetylated oxidized starch (INS 1451)* is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride

C.A.S numbers

Acetylated distarch phosphate (INS No. 1414)
9067-33-8(Modified starch)
68130-14-3(Modified starch)
113894-91-0 (Modified amylopectin)

Starch acetate (INS No. 1420)
9045-28-7 (Modified starch)

Acetylated distarch adipate (INS No. 1422)
63798-35-6(Modified starch)
63055-36-7 (Modified amylopectin)

Acetylated oxidized starch (INS No. 1451)

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68187-08-6(Modified starch)

CHARACTERISTICS

IDENTIFICATION

Specific reaction for Acetyl groups	Passes TEST See description under TESTS
Ester groups	Passes TEST See description under TESTS

PURITY

Acetyl groups	Not more than 2.5% on the dried basis See description under TESTS
Vinyl acetate	Not more than 0.1 mg/kg See description under TESTS

TEST

IDENTIFICATION TESTS

Specific reaction for acetyl groups

Principle

Acetate is liberated upon saponification of acetylated starch and converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure

Suspend about 10 g of the sample in 25 ml water. Add 20 ml of 0.4 M NaOH. After shaking for 1 h filter the starch off and evaporate the filtrate in an oven at 110°. Dissolve the residue in a few drops of water and transfer 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 M NaOH. The blue colour is more distinct when the original yellow colour of the

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reagents is removed with 1 drop of a 1 in 10 solution of hydrochloric acid.

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 groups in the product.

PURITY TESTS

Acetyl groups

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 M sodium hydroxide to a permanent pink end-point. Add 25.0 ml of 0.45 M 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 gelatinise). 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 M hydrochloric acid to the disappearance of the pink colour. Record the volume, in ml of 0.2 M hydrochloric acid required as S.

Perform a blank titration on 25.0 ml of 0.45 M sodium hydroxide, and record the volume, in ml, of 0.2 M hydrochloric acid required as B.

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

where

M is the molarity of hydrochloric acid solution; and

W is the weight of samp'

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ANNEX 5: ADDITIONAL SPECIFICATIONS FOR STARCHES SUBJECTED TO OXIDATION

(VERSION 2018 - TENTATIVE)

Information is required on:

- *A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method.*

APPLIES TO

Oxidized starch (INS No. 1404)

Acetylated oxidized starch (INS No. 1451)

TREATMENT

Sodium hypochlorite is used for oxidation.

- *Oxidized starch (INS 1404)* is obtained by treatment of food starch with sodium hypochlorite.
- *Acetylated oxidized starch (INS 1451)* is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride.

Oxidation involves the deliberate production of carboxyl groups.

C.A.S number

Oxidised starch (INS No. 1404)
65996-62-5 (modified starch)
113894-86-3 (modified amylopectin)

Acetylated oxidised starch (INS No. 1451)
68187-08-6

CHARACTERISTICS

IDENTIFICATION

Test for hypochlorite oxidized starch

Passes test

See description under TESTS

PURITY

Carboxyl groups (Vol. 4)

Not more than 1.3% on the dried basis

Residual hypochlorite

Information required

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TESTS

IDENTIFICATION TESTS

Test for hypochlorite oxidized 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. The test is not suitable for slightly oxidized potato starch due to the presence of phosphate groups.

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.

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ANNEX 6: ADDITIONAL SPECIFICATIONS FOR STARCHES ESTERIFIED WITH OCTENYLSUCCINIC ANHYDRIDE

Version 2018

APPLIES TO Starch sodium octenylsuccinate (INS No. 1450)

TREATMENT Octenylsuccinic anhydride can be used for the esterification and either sodium hydroxide or sodium carbonate as a pH buffer for neutralisation.

C.A.S numbers Starch sodium octenylsuccinate
66829-29-6(Modified starch)
52906-93-1(Modified starch)
125109-81-1 (Modified amylopectin)

CHARACTERISTICS

PURITY

Octenylsuccinyl groups Not more than 3% on the dried basis

See description under TESTS

Residual free octenylsuccinic acid *Not more than 0.3% on the dried basis*

See description under TESTS

PURITY TEST

Octenylsuccinate groups and residual free octenylsuccinic acid in Starch sodium octenyl succinate

Principle
Residual free octenylsuccinic acid in the sample is extracted and determined by HPLC/UV. Total octenylsuccinic content is determined using the same method after hydrolysis of the sample. Octenylsuccinate ester groups on the modified starch are calculated by subtraction of the residual free octenylsuccinic acid from the total.

Standard and Reagents

Octenylsuccinic anhydride:
2-Octen-1-ylsuccinic anhydride, mixture of *cis* and *trans* (>97%) (CAS: 42482-06-4)

0.1 N potassium hydroxide:
Weigh1.4 g of potassium hydroxide, dissolve in water and dilute to 250 ml

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0.073 mol/l phosphoric acid:

Dilute 1 ml of phosphoric acid (85%, density 1.686g/cm^3) to 200 ml with water.

Preparation of standard solutions

Accurately weigh about 20 mg of octenylsuccinic anhydride, add 10 ml of 0.1N potassium hydroxide, stopper and heat at 80° for 3 hours. After cooling, add 8 ml of 0.073mol/l phosphoric acid and dilute with water to 20 ml. Pipette 2 ml of this solution into a 20 ml volumetric flask and dilute with water. Pipette 1 ml, 2 ml, 5 ml, and 10 ml of the resulting solution into four separate 20-ml volumetric flasks, and dilute each to volume with water to prepare standards of 5 µg/ml, 10 µg/ml, 25 µg/ml and 50 µg/ml respectively.

Preparation of test solution A (for residual octenylsuccinic acid):

Accurately weigh about 0.1 g of sample, add 20 ml of methanol, and shake for 18 hours or more. Centrifuge the mixture at about 3000 rpm for 5 minutes, pipette 10 ml of the supernatant, and evaporate to dryness under vacuum at 40°. Dissolve the residue and dilute with water in a 5 ml volumetric flask.

Preparation of test solution B (for total octenylsuccinic acid):

Accurately weigh about 20 mg of sample, dissolve in 10 mL of 0.1N potassium hydroxide, stopper and heat at 80°for 3 hours. After cooling, add 8 ml of 0.05mol/l phosphoric acid, dilute with water to 20 ml.

Procedure

HPLC operating conditions

- Column: A octadecylsilanized silica gel column (250 mm x 4.6 mm, 5µm) (L-Column ODS-V CERI or equivalent)
- Column temperature: 40°
- Detector: UV at 205 nm
- Mobile phase: A 1:1 mixture of 0.1% (v/v) phosphoric acid solution / acetonitrile
- Injection volume: 20µl
- Flow rate: Adjust the retention time of the main peak to about 9 minutes.

Inject the test solution A and B and the standard solutions into an HPLC under the same conditions.

Measure the sum of the peak areas of two main peaks of *cis*- and *trans*-2-octenylsuccinic acid for each standard solution, and prepare a standard curve for octenylsuccinic anhydride from the sums obtained and the concentrations of octenylsuccinic

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anhydride in the standard solutions. Measure the sum of the peak areas of two main peaks for the test solutions A and B. Determine the concentration of octenylsuccinic acid (µg/ml) in the test solutions A and B from the standard curve, and calculate residual and total octenylsuccinic acid, respectively. The value of octenylsuccinate groups in the sample is calculated by the following formula:

Calculation:

$$Residual\ free\ octenylsuccinic\ acid\ \% = C_{OS}\ x\ 1.086\ /\ W_r\ x\ 100$$

$$Total\ octenylsuccinic\ acid\ \% = C_{os}\ x\ 1.086\ /\ W_s\ x\ 500$$

Where

- 1.086 is the molecular weight of octenylsuccinic acid divided by the molecular weight of octenylsuccinic anhydride
- C_{os} is the octenylsuccinic anhydride concentration (µg/ml);
- W_r or W_s is the dry-basis weight of the sample (g).

Content (%) of octenylsuccinyl groups =
Content of total octenyl succinic acid – Content of residual octenylsuccinic acid.

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ANNEX 7: ADDITIONAL SPECIFICATIONS FOR STARCHES ETHERIFIED WITH PROPYLENE OXIDE

(Version 2018 - TENTATIVE)

Information is required on:

- *A suitable method for the determination of propylene chlorohydrin with detection limit lower than 0.1 mg/kg and data on at least 5 representative batches of Hydroxypropyl starch using the method.*

APPLIES TO

Hydroxypropyl starch (INS No. 1440)

Hydroxypropyl distarch phosphate (INS No. 1442)

TREATMENT

Propylene oxide is used for etherification.

- *Hydroxypropyl starch (INS No. 1440)* is obtained by etherification of unmodified food starch with propylene oxide.
- *Hydroxypropyl distarch phosphate (INS No. 1442)* is obtained by esterification of unmodified 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.

C.A.S numbers

Hydroxypropyl starch(INS No. 1440)
9049-76-7(Modified starch)
74315-67-6 (Modified amylopectin)

Hydroxypropyl distarch phosphate (INS No. 1442)
53124-00-8(Modified starch)
113894-92-1 (Modified amylopectin)

CHARACTERISTICS

IDENTIFICATION

Hydroxypropyl ether groups

Passes test

See description under TESTS

PURITY

Hydroxypropyl groups

Not more than 7.0% on the dried basis

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See description under TESTS

Propylene chlorohydrins

Not more than 1 mg/kg

See description under TESTS

TESTS

IDENTIFICATION TESTS

Hydroxypropyl ether groups[1]

Ninhydrin reagent

A 3% solution of 1,2,3-triketohydrindene crystals in 5% 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 TEST

Hydroxypropyl groups

Ninhydrin reagent

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

[1] USP29-NF34: U.S. Pharmacopeial Convention, Hydroxylpropyl corn starch monograph, 2015. Reproduced from the USP-NF with permission from The U.S. Pharmacopeial Convention (USP)

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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

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

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 chlorohydrins

Principle

Propylene chlorohydrins (1-chloro-2-propanol and 2-chloro-1-propanol) in sample are determined by capillary gas chromatography.

Standards and Reagents

propylene chlorohydrins

Preparation of standard addition calibration curve

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Accurately weigh about 50 mg propylene chlorohydrins, and dilute with water to 100 ml. Dilute 10 ml of this solution to 100 ml with water to make a standard stock solution (50 µg/ml). Take four Erlenmeyer flasks and weigh 50.0 g of sample in each one. Add 125 ml of 1 M sulfuric acid in each one. Add 0.5 ml, 1 ml, 2 ml, or 5 ml of standard stock solution, to the 1st, 2nd, etc flasks respectively. Proceed as directed for the test solution, beginning with “and swirl the flask to disperse the contents,” to prepare the standard solutions of added concentration 5, 10, 20 and 50 µg/ml respectively.

Preparation of test solution

Accurately weigh 50 g of sample into an Erlenmeyer flask, add 125 ml of 1 M sulfuric acid, and swirl the flask to disperse the contents. Stopper loosely, heat in a water bath at 100°for 10 min, mix the contents well, and heat for an additional 30 min. For starches that are not easy to hydrolyze, such as wheat starch, heating time should be longer (90 min). Cool to room temperature, adjust the pH to 7 with 25% sodium hydroxide solution, and filter with suction through a glass-fiber filter paper. Wash the flask and the residue on the filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, stir for 5–10 min to dissolve, and transfer the solution into a separating funnel. Wash the flask with 25ml of water, and add the washings to the funnel. If precipitate remains, stir well with a small amount of water to dissolve it completely, and add the solution to the funnel. Extract five times with five 50ml portions of diethyl ether. Combine the diethyl ether extracts, add 3 g of anhydrous sodium sulfate, let it stand for a few minutes and filter through a filter paper. Wash the flask and the filter paper with 25 ml of diethyl ether, and combine the washings with the filtrate. Evaporate to 4 ml in a water bath at about 40° under atmospheric pressure, cool, transfer to a 5 ml volumetric flask and add diethyl ether to the exact volume.

Procedure

GC operating conditions

- GC equipped with a flame ionization detector (FID).
- Column: A fused silica column coated with polyethylene glycol (30 m x 0.25 mm i.d., 0.25 µm) (Inert Cap WAXGL Sciencesor equivalent）
- Carrier gas: N_2 or He

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- Flow rate: Adjust the retention time of 1-chloro-2-propanol to about 15 min
- Column temperature: 40°-for 2 min; heat at 5°/min to 80°, keep for 8 min, heat at 25°/min to 230°, keep for 5 min
- Injector temperature: 150°
- Detector temperature: 230°
- Split-less（purge start: 1 min after injection）

Analyse 1-μl portions of the test solution and the standard solutions by gas chromatography, using the operating conditions given above. Prepare a standard addition curve: Plot in the y axis the sum of the peak areas corresponding to 1-chloro-2-propanol and 2-chloro-1-propanol in the chromatographs and in the x-axis the added concentration of propylene chlorohydrins in the standard solution. For the test solution the added concentration is equal to 0. A linear calibration curve should be obtained. Extrapolate to 0 in the y axis. The concentration (μg/ml) of propylene chlorohydrins in the test solution is equal to the absolute value of the concentration at the point where the curve intercepts the x axis (C_t). Determine the content of propylene chlorohydrins in the sample using the following formula:

Calculation

$$\text{Content (mg/kg) of Propylene chlorohydrins} = C_t \times 5 / W$$

where

- C_t: amount of propylene chlorohydrins in test solution (μg/mL);
- W: mass of sample (g, on the dried weight basis)

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ANNEX 8: ADDITIONAL SPECIFICATIONS FOR STARCHES CROSSLINKED WITH ADIPIC ANHYDRIDE

(Version 2018 – Tentative)

Information is required on:

- *A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches*
- *Levels of free adipic acid in at least 5 representative batches*

APPLIES TO Acetylated distarch adipate (INS No. 1422)

TREATMENT Adipic anhydride can used for esterification and crosslinking. In cases of cross-linking, where adipic anhydride connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = $CO-(CH_2)_4-CO$ and starch refers to the linear and/or branched structure.

C.A.S numbers Acetylated distarch adipate
63798-35-6(Modified starch)
63055-36-7 (modified amylopectin)

CHARACTERISTICS

PURITY

Adipate groups Not more than 0.135% on the dried basis

See description under TESTS

Free adipic acid *Information Required*

See description under TESTS

PURITY TEST

Adipate groups and free adipic acid Determine by gas chromatography after derivatization

Principle
Free adipic acid in the sample is extracted and determined by capillary gas chromatography after trimethylsilyl-derivatization. Total adipic acid is determined using the same method after

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hydrolysis of the sample and adipate groups are calculated by subtraction of the free adipic acid from the total.

Standards and Reagents
Adipic acid (>99%)
Glutaric acid (>99%)
Starch, unmodified (of the same botanical origin as the sample)
Sodium hydroxide solution (4N): weigh 40g of NaOH, dissolve in water and dilute to 250 ml.
Concentrated HCl (36%)
Ethyl acetate
Sodium sulphate, anhydrous
N,O-Bis(trimethylsilyl)trifluoroacetamide
Pyridine

Internal standard solution (1 mg/ml)
Accurately weigh 0.1 g of glutaric acid, dissolve in water and dilute to 100 ml.

Standard stock solution (1 mg/ml)
Accurately weigh 0.1 g of adipic acid, dissolve in 90 ml of warm water, cool to room temperature, dilute to 100 ml and mix.

Working standard solutions (0.02, 0.1, 0.2 and 0.4 mg/ml)
Pipette 1, 5, 10, and 20 ml of the standard stock solution in four separate 50 ml volumetric flasks, and dilute with water.

Procedure
Preparation standard curve solutions
Weigh 1.0 g of starch into each of four Erlenmeyer flasks, add 50 ml of water and 1 ml of internal standard solution. Add 5 ml each of the four working standard solutions, respectively. Stopper the flask and shake them well to disperse the starch, add 50 ml of 4N sodium hydroxide solution, and shake for 5 min. Place the flasks in a water bath, at room temperature, and add cautiously 20 ml of conc. hydrochloric acid. Cool, and quantitatively separately transfer the contents of the flasks into four separation funnels with a little amount of water. Extract three times with 100 ml of ethyl acetate each time. Collect the ethyl acetate layers separately in four dry Erlenmeyer flasks, add 20 g of anhydrous sodium sulphate, allow to stand for 10 min with occasional shaking, and filter into a rotary evaporator flask. Wash the Erlenmeyer flask and the residue on the filter paper twice with a small quantity of ethyl acetate, and combine the washings with the filtrate. Evaporate the ethyl acetate under a reduced pressure of 6.7 kPa at a temperature below 40°. Remove the remaining ethyl acetate completely by nitrogen stream. The evaporation of ethyl acetate should be effected as quickly as possible. Successively add 2 ml of pyridine and 1 ml of N,O-bis(trimethylsilyl)trifluoroacetamide to the residue and stopper the

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flask. Allow the solution to stand for 1 hour, transfer 2 ml of it into a GC vial, and immediately stopper tightly. Use these solutions to construct standard curve (Internal standard 1 mg/g starch, standards 0.1, 0.5, 1 and 2 mg/g starch respectively)

Preparation of test solution A (for residual free adipic acid)
Weigh accurately about 5 g of sample into an Erlenmeyer flask, add 100 ml of water and 1 ml of the internal standard solution. Shake well for 1 hour, and filter through a 0.45 μm membrane filter. To the filtrate, add exactly 1 ml of hydrochloric acid (in the case of pre-gelatinized starch or water-soluble starch, directly add 1 ml of hydrochloric acid to the resulting suspension without filtering), and transfer into a separation funnel. Proceed as directed for the preparation of standard solutions, beginning with "…and wash the inside of the flask with a little amount of water into the funnel." Use this solution for the determination of residual free adipic acid (Internal standard 1 mg/ 5 g starch).

Preparation of test solution B (for total adipic acid)
Weigh accurately about 1 g of sample into an Erlenmeyer flask, add 50 ml of water and exactly 1 ml of the internal standard solution. Shake the mixture well to disperse the starch, add 50 ml of 4N sodium hydroxide solution and shake well for 5 minutes. Place the flask in a water bath at room temperature, and add cautiously 20 ml of concentrated hydrochloric acid. After cooling, transfer the contents in the flask into a separation funnel. Proceed as directed for the preparation of standard solution, beginning with "…and wash the inside of the flask with a little amount of water into the funnel." Use this solution for the determination of total adipic acid (Internal standard 1 mg/g starch).

Procedure
GC operating conditions

- GC equipped with a flame ionization detector (FID) Column:A fused silica column coated with a mixture of 50% diphenyl and 50% dimethylpolysiloxane (15 m x 0.25 mm i.d., 0.25 μm)
- Carrier gas: He
- Column flow 1.0 ml/min.
- Column temperature: 120°-5 min-5°/min-150° (Glutaric and adipic acids elute at about 5 min and 8 min respectively)
- Injector temperature: 250°
- Detector temperature: 250°
- Injection volume 1µl
- Split ratio: 30:1

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Inject standard curve solutions into the capillary GC under the conditions indicated and construct a standard curve using the peak area ratios of adipic acid and glutaric acid against the amounts of adipic acid in the standard solutions (in g). Inject the test solution A and Band obtain the peak area ratio of adipic acid to glutaric acid for each of the test solutions A and B.

Determine the amount of adipic acid in each test solution from the standard curve and calculate the percent of adipate groups using the following formula:

Free adipic acid, %w/w = [CF/MF] x 100

Adipate groups. %w/w = [CT/MT–CF/MF] x 100

where

CT = amount of the total adipic acid in the test solution B (g)
CF = amount of the free adipic acid in the test solution A (g)
MT =mass of sample in the test solution for the determination of total adipic acid (g, on the dried weight basis)
MF =mass of the sample in the test solution for the determination of free adipic acid (g, on the dried weight basis)

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