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# Acetylated Distarch Adipate

(Tentative)

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# ACETYLATED DISTARCH ADIPATE
(TENTATIVE)

*Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Acetylated distarch adipate 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 test for identification of the adipate groups*
- *Levels of free adipic acid*

**SYNONYMS** INS No. 1422

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.

Acetylated distarch adipate is a modified starch. It is obtained by esterification of food starch with acetic anhydride and esterification/cross-linking with adipic anhydride, in accordance with good manufacturing practice. Acetylation results in substitution of hydroxyl groups with acetyl esters. 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-(CH2)4-CO and Starch refers to the linear and/or branched structure.

Acetylated distarch adipate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 63798-35-6
63055-36-7 (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<br>See description under TESTS |
| Iodine stain | Passes test<br>See description under TESTS |
| Copper reduction | Passes test<br>See description under TESTS |
| Specific reaction for acetyl groups | Passes test<br>See description under TESTS |
| Ester groups | Passes test<br>See description under TESTS |
| Test for adipate groups | *Information Required* |

PURITY

| | |
|---|---|
| Loss on drying (Vol. 4) | Cereal starch: not more than 15.0%<br>Potato starch: not more than 21.0%<br>Other starches: not more than 18.0%<br>(120°, 4 h, vacuum not exceeding 100 mm Hg) |
| Acetyl groups | Not more than 2.5% on the dried basis<br>See description under TESTS |
| Adipate groups | Not more than 0.135% on the dried basis<br>See description under TESTS |
| Free Adipic Acid | *Information Required*<br>See description under TESTS |
| Sulfur dioxide (Vol. 4) | Not more than 50 mg/kg on the dried basis for modified cereal starches<br>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<br><br>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<br><br>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 |
| Specific reaction for acetyl groups | Principle<br>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.<br><br>Procedure<br>About 10 g of the sample is suspended in 25 ml water to which is added 20 ml of 0.4 M 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 M 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. |

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 or adipyl groups in the product.

Test for adipate groups

*Information Required*

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 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 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.

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

where

M is the molarity of hydrochloric acid solution; and
W is the weight of sample, In grams.

Adipate groups and Free Adipic Acid

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 (%).