USDA Foreign Agricultural Service
GAIN Report
Global Agriculture Information Network
Template Version 2.09

Required Report - Public distribution

Date: 2/20/2008

GAIN Report Number: JA8008

Japan

Food and Agricultural Import Regulations and Standards

Designation of Modified Starches as a Food Additive

2008

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Report Highlights:
On February 15, 2008, MHLW announced designation of Modified Starches as a food additive.

Includes PSD Changes: No
Includes Trade Matrix: No
Annual Report
Tokyo [JA1]
[JA]

Executive Summary

On February 15, 2008, MHLW announced designation of Modified Starches as a food additive.

Purpose

The period for comments directly to MHLW will close February 29, 2008. However MHLW will also notify these proposed changes to the WTO/SPS committee, which would be the last chance for public comments to be submitted on this subject. Then after the closing of a the comment period in the WTO, a report to the Minister of Health, Labour, and Welfare will be made based on the conclusions of a session of the Pharmaceutical Affairs and Food Sanitation Council slated to be held at a later date, and this will constitute the final decision.

If you have comments that you would like to be considered for inclusion in the official U.S. Government comments to the MHLW, please send those as soon as possible to the Agriculture Section of the U.S. Embassy in Tokyo, at agtokyo@usda.gov.

For comments directly to MHLW, please send those to following contacts.

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Outline

The Ministry of Health, Labour and Welfare is going to newly designate 11 modified starches as authorized food additives.

Under Article 10 of the Food Sanitation Law, food additives can be used or marketed only when they are designated by the Minister of Health, Labour and Welfare. When use standards or compositional specifications are established for food additives, based on Article 11 of the law, those additives are not permitted to be marketed unless they meet these standards or specifications.

In response to a request from the Minister, the Subcommittee on Food Additives under the Food Sanitation Committee under the Pharmaceutical Affairs and Food Sanitation Council has discussed the adequacy of the designation of these substances. The subcommittee has concluded as follows.

Conclusion from the subcommittee

The Minister should designate the 11 modified starches given below, based on Article 10 of the Food Sanitation Law, as a food additive unlikely to harm human health and establish compositional specifications for the substance, based on Article 11 of the law (see Attachment 2-1).

<Modified starches>
Acetylated distarch adipate, Acetylated distarch phosphate, Acetylated oxidized starch, Starch sodium octenyl succinate, Starch acetate, Oxidized starch Hydroxypropyl starch, Hydroxypropyl distarch phosphate, Phosphated distarch phosphate, Monostrach phosphate, Distarch phosphate

Additional Information
–Treatments and Status of Modified Starches (Attachment 2-2)
–Progress in the designation procedure of food additives that have been proven safe by JECFA (Joint FAO/WHO Expert Committee on Food Additives) and that are widely used in countries other than Japan (Attachment 2-3)

Attachment 2-1

Modified starches (11)

Acetylated Distarch Adipate
Acetylated Distarch Phosphate
Acetylated Oxidized Starch
Starch sodium Octenyl Succinate
Starch Acetate
Oxidized Starch
Hydroxypropyl Starch
Hydroxypropyl Distarch Phosphate
Phosphated Distarch Phosphate
Monostrach Phosphate
Distarch Phosphate

1. Standards for use
Not established.

2. Compositional specifications
See below.

Acetylated Distarch Adipate

Substance name Acetylated Distarch Adipate

Definition Acetylated Distarch Adipate is obtained by esterifying starch with acetic anhydride and adipic anhydride.

Description Acetylated Distarch Adipate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification

(1) Add a few drops of iodine TS to a suspension of Acetylated Distarch Adipate (1 in 10). A dark blue to red color develops.

(2) Suspend 2.5 g of Acetylated Distarch Adipate by adding 10 ml of diluted hydrochloric acid (2 in 19) and 70 ml of water, and heat under a reflux for about 3 hours. After cooling, add 5.0 ml of the resulting suspension to 5 ml of boiling Fehling's TS. A red precipitate develops.

(3) To 0.5 g of Acetylated Distarch Adipate, add 10 ml of sodium carbonate TS, boil for 3 minutes, and add 10 ml of dilute sulfuric acid. An odor of acetic acid is emitted.

Purity (1) Adipate groups Not more than 0.135%.

(i) *Test Solution for Determination of Total Adipic Acid* Weigh accurately about 1 g of Acetylated Distarch Adipate into an Erlenmeyer flask, and add 50 ml of water and 1 ml of the internal standard solution. Shake the mixture well to disperse the starch, add 50 ml of sodium hydroxide solution (4 in 25), and shake well for 3 minutes. Place the flask into a water bath of room temperature, and add 20 ml of hydrochloric acid cautiously. After cooling, transfer the content in the flask into a separating funnel, wash the flask with a little amount of water into the funnel, and add the washings to the funnel. Extract three times with 100 ml of ethyl acetate each time, and collect the ethyl acetate layers in a flask. Add 20 g of anhydrous sodium sulfate, allow to stand for 20 minutes with occasional shaking, and filter. Wash the flask and the residue on the filter paper twice with 50 ml of ethyl acetate each time, and combine the washings with the filtrate. Evaporate the ethyl acetate under the vacuum pressure of 6.7 kPa at a temperature below 40°C. 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, stopper, and dissolve it. Allow the solution to stand for 1 hour, transfer 2 ml of it into a glass vial, and immediately stopper tightly.

Internal Standard Solution Weigh accurately 0.10 g of glutaric acid, add water, and dissolve and making up to 100 ml.

(ii) *Test Solution for Determination of Free Adipic Acid* Weigh accurately about 5 g of Acetylated Distarch Adipate into an Erlenmeyer flask, and add 100 ml of water and 1 ml of the internal standard solution. Shake well for 1 hour and filter through a membrane filter (0.45-μm pore size). To the filtrate, add exactly 1 ml of hydrochloric acid (if the sample is pregelatinized starch, directly add 1 ml of hydrochloric acid to the resulting suspension, without filtering), and transfer the content into a separating funnel. Then proceed as directed for the test solution for determination of the total adipic acid.

(iii) *Standard Solutions* Add water to 0.10 g of adipic acid, exactly weighed, and dissolve and make up to exactly 100 ml. Place exactly 1 ml, 5 ml, 10 ml and 20 ml of this solution in four 50-ml volumetric flasks, respectively, and make up with water exactly to volume. Use the four solutions as the standard stock solutions. Weigh 1.0 g of unmodified starch (the same botanical origin as the test substance) into each of 4 Erlenmeyer flasks, and add 50 ml of water and 1 ml of the internal standard solution to each. Then add 5 ml of the standard stock solutions, respectively. Shake them well to disperse the starch, add 10 ml of sodium hydroxide solution (4 in 25), and shake for 5 minutes. Place the flasks in a water bath of room temperature, and add cautiously 20 ml of hydrochloric acid. Cool, and separately transfer the contents of the flasks into separating funnels. Then prepare four standard solutions in the same manner as for the test solution for determination of the total adipic acid.

(iv) *Procedure* Analyze 1 μl portions of the test solution for determination of total adipic acid, the test solution for determination of free adipic acid, and the standard solutions by Gas Chromatography using the operating conditions below. Obtain the peak area ratio of adipic acid to glutaric acid for each standard solution. Prepare a calibration curve from the peak area ratios and the concentrations of adipic acid in the standard solutions. Obtain the peak area ratio of adipic acid to glutaric acid for each of the two test solutions, and calculate the adipic acid concentration in each test solution from the calibration curve. Determine the content of adipic acid groups from the following formula.

Content (%) of adipate groups

$$= \left[\frac{C_T}{W_T} - \frac{C_F}{W_F}\right] \times 800$$

W_T: Dry basis weight of the sample in the test solution for determination of the total adipic acid (g)

W_F: Dry basis weight of the sample in the test solution for determination of the free adipic acid (g)

C_T: Adipic acid concentration in the test solution for determination of the total adipic acid (g/ml)

C_F: Adipic acid concentration in the test solution for determination of the free

adipic acid (g/ml)

Operating Conditions

Detector: Flame ionization detector

Detector temperature: 250°C

Column: A silicate glass capillary (15-m length and 0.25-mm internal diameter) coated with a mixture of 50% diphenyl and 50% dimethylpolysiloxane at 0.25-µm thickness

Column temperature: Maintain the temperature at 120°C for 3 minute, and raise to 150°C at 5°C/minute

Injection port temperature: 250°C

Injection: Split (30:1)

Carrier gas: Helium or nitrogen

Flow rate: Adjust so that the retention times of adipic acid and glutaric acid are about 2 minutes and about 3 minutes, respectively.

(2) Acetyl groups Not more than 2.5%

Test Solution: Weigh accurately about 5 g of Acetylated Distarch Adipate into an Erlenmeyer flask, and add 50 ml of water to disperse. (If the sample is pregelatinized starch or water-soluble starch, use 100 ml of water). Add a few drops of phenolphthalein TS, add dropwise 0.1 mol/L sodium hydroxide until a pale pink color develops. Add exactly 25 ml of 0.45 mol/L sodium hydroxide, close the stopper, and agitate for 30 minutes. Remove the stopper, and wash the ground-glass joints and internal surfaces with a little amount of water into the flask.

Procedure: Titrate the excess sodium hydroxide with 0.2 mol/L hydrochloric acid, and record the volume consumed as *B* (ml). The endpoint is when the pale pink color disappears. Separately conduct a blank test by titrating 25 ml of 0.45 mol/L sodium hydroxide with 0.2 mol/L hydrochloric acid and recording the volume consumed as B (ml). Obtain the content of acetyl groups by the following formula.

$$\text{Content (\%) of acetyl groups (CH}_3\text{CO-)} = \frac{(B - S) \times 0.2 \times 0.043}{\text{Dry-basis weight of the sample (g)}} \times 100$$

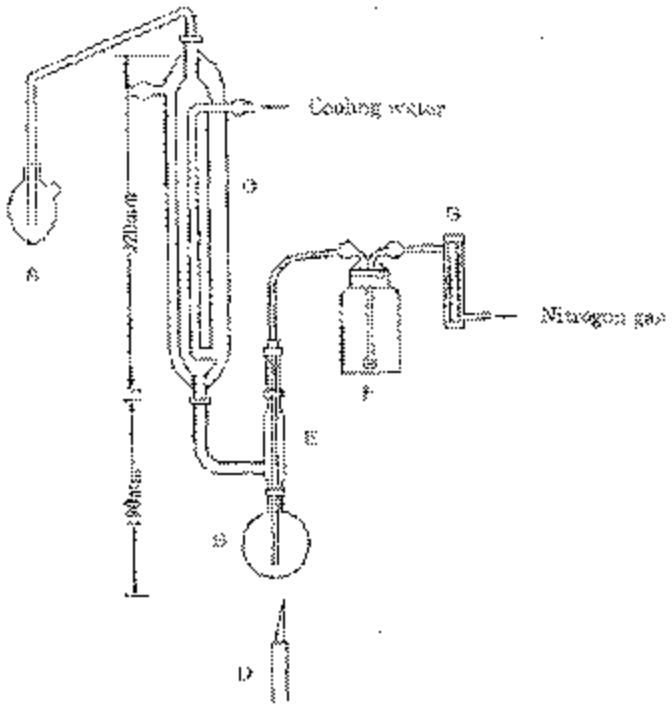
(3) Lead Not more than 2.0 µg/g as Pb (5.0 g, Method 1)

(4) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 2, Apparatus B)

(5) Sulfur dioxide Not more than 50 µg/g

(6) Apparatus

Use the apparatus illustrated in the figure.



A: 50-ml pear shaped flask
B: 100-ml round bottom flask
C: Double condenser
D: Micro burner
E: Glass capillary
F: Scrubber
G: Flow rate meter

(ii) Operation

Test Solutions A and B Assemble the apparatus, and connect flask A containing 20 ml of 0.1 mol/L sodium hydroxide. Attach Flask B containing 20 ml of distilled water, 1 ml of dimedone TS, 1 ml of sodium azide (1 in 100), 2 ml of ethanol, 2 drops of silicone resin, and 10 ml of diluted phosphoric acid (1 in 4). Pass nitrogen gas at 0.5–0.6 L/minute for 3 minutes through meter G. Partially detach flask B, promptly place exactly 2.0 g of Acetylated Distarch Adipate in it, and connect again. Adjust the height of burner D 4–5 cm, and heat flask B for about 10 minutes, passing nitrogen gas at 0.5–0.6 L/minute. Remove the flask, and use the solution inside as the sample solution. Measure exactly two 5-ml portions of the test solution, and add 1.0 ml of water to one and

0.1 ml of 0.3% hydrogen peroxide to the other. Refer to the former as solution A and the latter as solution B. To each solution, add 1 ml of *p*-toranaline–formaldehyde TS, shake well, and allow to stand at room temperature for 15 minutes. Use them as test solutions A and B.

Standards Solutions Weigh exactly 0.1623 g of sodium hydrogen sulfite, and dissolve in 0.1 mol/L sodium hydroxide to make 100 ml. Measure exactly 1 ml of the this solution, dilute with 0.1 mol/L sodium hydroxide to 100 ml. Place 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml of the resulting solution in five 25-ml volumetric flasks, respectively, and add 0.1 mol/L sodium hydroxide to make exactly 25 ml of each. Use them as the standard stock solutions. Measure two 5-ml portions of each of the stock solutions, and add 1.0 ml of water and 0.1 ml of 0.3% hydrogen peroxide, respectively in the same manner as for the test solutions. Prepare solutions A and B for each concentration.

Procedure Measure the absorbances of the test solutions A and B against 0.1 mol/L sodium hydroxide at 560 nm, and express A_A and A_B, respectively. Record the value ($A_A - A_B$) as the absorbance of the test solution. Then obtain the value ($A_A - A_B$) of each of the standard solutions in the same manner, and prepare a calibration curve. Obtain sulfur dioxide concentration (μg/ml) in the test solution from the calibration curve, and calculate the content (μg/g) by the following formula.

$$\text{Content (μg/g) of sulfur dioxide} = \frac{\text{Sulfur dioxide concentration in the test solution (μg/ml)} \times 20}{\text{Dry-basis weight of the sample (g)}}$$

Loss on Drying Not more than 21.0% (120°C, not more than 15.3kPa, 4 hours).

Acetylated Oxidized Starch

Substance name Acetylated Oxidized Starch

CAS number [68187-08-6]

Definition Acetylated Oxidized Starch is obtained through treatment of starch with sodium hypochlorite, followed by esterification with acetic anhydride.

Description Acetylated Oxidized Starch occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

(3) Proceed as directed in Identification (5) for Acetylated Distarch Adipate.

(4) Carboxyl groups Suspend 0.05 g of Acetylated Oxidized Starch in 25 ml of a solution of methylene blue in methanol (1 in 100), and allow to stand for 5–10 minutes with occasional shaking. Remove the supernatant by decantation, wash the precipitate with water, and examine using an optical microscope. Dark blue starch granules are observed.

If the sample is pregelatinized starch, suspend 0.05 g of Acetylated Oxidized Starch in 25 ml of 1% methylene blue solution, and allow to stand one night. Remove the supernatant by decantation, wash the precipitate with water, and examine them using an optical microscope. Dark blue starch granules are observed.

Purity (1) Acetyl groups Not more than 2.5%.
Proceed as directed in Purity (2) for Acetylated Distarch Adipate.

(2) Carboxyl groups Not more than 1.5%

If necessary, grind the sample with care to prevent moisture, sieve through an 840-μm standard sieve, and mix completely. Weigh exactly 5.00 g of Acetylated Oxidized Starch into a beaker, add 25 ml of 0.1 mol/L hydrochloric acid, allow to stand for 30 minutes with occasional shaking, and filter under suction. Transfer the residue in the beaker into the filter with the aid of water, and wash the residue on the filter with water until the washings are free of chloride. Transfer the residue into a beaker, add 300 ml of water to suspend, and heat in a water bath with stirring to gelatinize and heat additional 15 minutes. Put the beaker out of the water bath, and titrate with 0.1 mol/L sodium hydroxide while hot. Record the volume consumed as S (ml). Use 3 drops of phenolphthalein TS as the indicator. Separately, weigh the equal amount of the sample into a beaker, add 10 ml of water to suspend, and stir for 30 minutes. Filter the suspension under suction, transfer the residue in the beaker into the filter with the aid of water, and wash the residue on the filter paper with 200 ml of water. Suspend the residue in 300 ml of water, proceed as directed for the test above, and record the volume consumed as B (ml). If the sample is a pregelatinized starch, use 80% (v/v) ethanol solution (9 in 1,000), instead of 0.1 mol/L hydrochloric acid in the test and instead of water in the blank test. Also, if necessary, use a filter holder for filtering under suction. Obtain the content of carboxyl groups by the formula.

$$\text{Content (\%) of carboxyl groups (-COOH)} = \frac{(S-B)\times 0.0045}{\text{Dry basis weight of the sample (g)}}\times 100$$

If the sample is originated from of potato starch, the correction should be made as directed below since native phosphate groups present in potato starch increase the time obtained in this method.

Correction Obtain the phosphorous content (%) according to Purity (3) for

Acetylated Distarch Phosphate: calculate the deduction (%) by the following formula, and deduce the percentage from the content of carbonyl groups.

$$\text{Deduction (\%)} = \frac{2 - 43.02 \times P}{30.97}$$

(3) Lead: Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(4) Arsenic: Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(5) Sulfur dioxide: Not more than 50 μg/g.

Proceed as directed in Purity (6) for Acetylated Distarch Adipate.

Loss on Drying: Not more than 21.0% (130°C, not more than 13.3 kPa, 4 hours).

Acetylated Distarch Phosphate

Substance name: Acetylated Distarch Phosphate

CAS number: [68130-14-3]

Definition: Acetylated Distarch Phosphate is obtained by esterifying starch with sodium trimetaphosphate or phosphorus oxychloride, and acetic anhydride or vinyl acetate.

Description: Acetylated Distarch Phosphate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification: (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

(3) Proceed as directed in Identification (3) for Acetylated Distarch Adipate.

Purity: (1) Acetyl groups: Not more than 2.5%.

Proceed as directed in Purity (2) for Acetylated Distarch Adipate.

(2) Vinyl acetate: Not more than 0.1 μg/g.

Test Solution: Weigh an amount of Acetylated Distarch Phosphate equivalent to 5.0 g on the dried basis in a 20-ml vial (a vial designed for headspace gas chromatography) containing a stirring bar. Add exactly 5 ml of water, stopper tightly, and stir for 20 minutes.

Standard Solution: Weigh exactly 0.05 g of vinyl acetate, dissolve it in water, and make exactly 100 ml. Measure 1 ml of this solution, and dilute with water to exactly 100 ml. Then dilute exactly 2 ml of the resulting solution with water to make exactly 100 ml.

Use the obtained solution as the standard stock solution. Place 5 ml of the standard stock solution into a 20-ml vial (designed for headspace gas chromatography) containing a stirring bar and unmodified starch (the same botanical origin as the test substance) equivalent to 5 g on the dried basis, and stopper tightly. Stir for 20 minutes.

Procedure—Analyze the test solution and the standard solution by Headspace Gas Chromatography using conditions given below. The peak area of vinyl acetate for the test solution does not exceed that for the standard solution.

Operating conditions

Detector: Flame ionization detector

Detector temperature: 250°C

Column: A silicate glass capillary (10-m length and 0.25-mm internal diameter) coated with styrene-divinylbenzene polymer at 3-μm thickness

Column temperature: A constant temperature of about 110°C

Injection port temperature: 200°C

Injection: Split (10:1)

Carrier gas: Nitrogen

Flow rate: Adjust so that the peak of vinyl acetate appears about 9 minutes after injection.

Headspace sampler

Equilibrium temperature in the vial: 70°C

Equilibrium time in the vial: 30 minutes

Injection line temperature: 80°C

Injection mount: 1.0 ml

(3) Phosphorous—Not more than 0.14% as P

Test Solution—Weigh accurately about 10 g of Acetylated Distarch Phosphate into an evaporating dish, and evenly sprinkle 10 ml of zinc acetate TS to the sample. Carefully evaporate to dryness on a hot plate, and increase the heat to carbonize the sample. Ignite it in a muffle furnace at 550°C for 1–2 hours until the ash is free from carbon, and cool. Add 15 ml of water, and wash down the inner surfaces with 5 ml of diluted nitric acid (1 in 3). Heat to boiling, cool, transfer the mixture into a 200-ml volumetric flask, wash the dish with three 20-ml portions of water, adding the washings to the flask, and add water to make 200 ml. Transfer an exactly measured aliquot (V ml) of this solution, containing phosphorous (P) not exceeding 1.5 mg, into a 100-ml volumetric flask, and add 10 ml of diluted nitric acid (1 in 6), 10 ml of ammonium vanadate TS, and 10 ml of ammonium molybdate TS for modified starch, mixing thoroughly after each addition. Dilute with water to exactly 100 ml, and allow to stand for 10 minutes.

Standard Solutions—Measure exactly 10 ml of Monopotassium Phosphate Standard Solution, and dilute to exactly 100 ml with water. Place 5 ml, 10 ml, and 15 ml of this solution in 100-ml volumetric flasks, respectively. To each of them, add 10 ml of diluted

nitric acid (1 in 3), 10 ml of ammonium vanadate TS, and 10 ml of ammonium molybdate TS for modified starch, mixing thoroughly after each addition. Dilute with water to exactly 100 ml, and allow to stand for 10 minutes.

Procedure Measure the absorbance of each of the test solution and the standards solutions at 460 nm, using the reference prepared as follows: To a 100-ml volumetric flask, add 10 ml of diluted nitric acid (1 in 3), 10 ml of ammonium vanadate TS, and 10 ml of ammonium molybdate TS for modified starch, mixing thoroughly after each addition, dilute with water to exactly 100 ml, and allow to stand for 10 minutes.

Determine the concentration of phosphorous in the test solution from the calibration curve, and calculate the content by the following formula.

$$\text{Content (\%) of phosphorus (P)} = \frac{\text{Phosphorous concentration in the test solution (mg/ml)} \times 2{,}000}{V \times \text{Dry-base weight of the sample (g)}}$$

(4) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1)

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.25 g, Method 3, Apparatus B).

(6) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (120°C, not more than 18.3 kPa, 4 hours).

Starch Sodium Octenyl Succinate

Substance name Starch Sodium Octenyl Succinate

Definition Starch Sodium Octenyl Succinate is obtained by esterifying starch with octenyl succinic anhydride.

Description Starch Sodium Octenyl Succinate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Purity (1) Residual octenyl succinic acid Not more than 0.8%.

Test Solution Weigh accurately about 0.1 g of Starch Sodium Octenyl Succinate, and add 25 ml of methanol, and shake for 12 hours or more. Centrifuge the mixture at about 3,000 rpm for 5 minutes, measure exactly 10 ml of the supernatant, and evaporate to dryness under vacuum at 40°C. Dissolve the residue by adding water and

make exactly 5 ml.

Standard Solutions Weigh accurately about 0.02 g of octeny succinic anhydride, add 10 ml of 0.1 mol/L potassium hydroxide, heat at 80°C for 2 hours. After cooling, add 8 ml of diluted phosphoric acid (1 in 200), and dilute with water to exactly 20 ml. Measure exactly 2 ml of this solution, and add water to make exactly 20 ml. Place 1 ml, 2 ml, 5 ml, and 10 ml of the resulting solution into separate 20-ml volumetric flasks, and dilute with water to exactly 20 ml.

Procedure Analyze 20 µl portions of the test solution and the standard solutions by Liquid Chromatography using the operating conditions given below. Measure the sum of the areas of two main peaks for each standard solution, and prepare a calibration curve for octenyl succinic anhydride. Measure the sum of areas of two main peaks for the test solution. Determine the concentration of octenyl succinic anhydride (µg/ml) in the test solution from the calibration curve, and calculate residual octenyl succinic acid in the sample product by the following formula.

$$\text{Content (\%) of residual octenyl succinic acid } (C_{12}H_{20}O_4) = \frac{\text{Octenyl succinic anhydride concentration } (\mu g/ml) \times 1.086}{\text{Dry-basis weight of the sample } (g) \times 1,000}$$

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 205 nm)

Column: A stainless steel tube of 25-cm length and 4.6-mm internal diameter

Packing material: 5-µm octadecylsilanized silica gel

Column temperature: 40°C

Mobile phase: A 1:1 mixture of phosphoric acid (1 in 1,000)/acetonitrile

Flow rate: Adjust so that the retention time of the main peak is about 6 minutes.

(2) Octenyl succinic groups Not more than 3%.

Test Solution Weigh accurately about 0.02 g of starch Sodium Octenyl Succinate, dissolve it in 10 ml of 0.1 mol/L potassium hydroxide, stopper, and heat at 80°C for 2 hours. After cooling, add 2 ml of diluted phosphoric acid (1 in 200), dilute with water to exactly 20 ml. Analyze the test solution by Liquid Chromatography using operating conditions directed in Purity (1). Measure the sum of the peak areas of the main two peaks, and determine the concentration of octenyl succinic anhydride (µg/ml) in the test solution from the calibration curve. Calculate the content (%) of the total octenyl succinic acid in the sample product by the following formula, and obtain the content (%) of octenyl succinic groups.

$$\text{Content (\%) of residual octenyl succinic acid } (C_{12}H_{20}O_4) = \frac{\text{Octenyl succinic anhydride concentration } (\mu g/ml) \times 1.086}{\text{Dry-basis weight of the sample } (g) \times 500}$$

Content (%) of octenyl succinic groups
= Content of total octenyl succinic acid
− Content of residual octenyl succinic acid

(3) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(5) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (130°C, not more than 18.0 kPa, 4 hours).

Starch Acetate

Substance name Starch Acetate

CAS number [9045-28-7]

Definition Starch Acetate is obtained by esterifying starch with acetic anhydride or vinyl acetate.

Description Starch Acetate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

(3) Proceed as directed in Identification (3) for Acetylated Distarch Adipate.

Purity (1) Acetyl groups Not more than 2.5%.

Proceed as directed in Purity (2) for Acetylated Distarch Adipate.

(2) Vinyl acetate Not more than 0.1 μg/g.

Proceed as directed in Purity (2) for Acetylated Distarch Phosphate.

(3) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(5) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (130°C, not more than 13.3 kPa, 4 hours).

Oxidized Starch

Substance name Oxidized Starch

Definition Oxidized Starch is obtained by treating starch with sodium hypochlorite.

Description Starch Acetate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

(3) Carboxyl groups Proceed as directed in Identification (4) for Acetylated Oxidize Starch.

Purity (1) Carboxyl groups Not more than 1.1%.
Proceed as directed in Purity (2) for Acetylated Oxidize Starch.

(2) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (120°C, not more than 13.3 kPa, 4 hours).

Hydroxypropyl Distarch Phosphate

Substance name Hydroxypropyl Distarch Phosphate

CAS number [53124-00-8]

Definition Hydroxypropyl Distarch Phosphate is obtained through esterification of starch with sodium trimetaphosphate or phoshporus oxychloride, followed by etherification with propylene oxide.

Description Hydroxypropyl Distarch Phosphate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate

Purity (1) Hydroxypropyl groups Not more than 7.0%.

Test Solution Weigh accurately about 0.1 g of Hydroxypropyl Distarch Phosphate, and add 25 ml of 0.5 mol/L sulfuric acid, and heat in a water bath to dissolve. After cooling, add water to make exactly 100 ml. If necessary, dilute it to assure the presence of less than 4 mg/100 ml of hydroxypropyl groups. Use the resulting solution as the sample solution. Measure exactly 1 ml of the sample solution into a graduated test tube, and add dropwise 8 ml of sulfuric acid with the tube immersed in cold water. Mix well, heat in a water bath for exactly 3 minutes, and immediately cool in ice water until the solution is cooled to room temperature. Add 0.6 ml of ninhydrin TS for modified starch, carefully allowing the reagent to run down the wall of the test tube. Immediately shake well, place in a 35°C water bath for 100 minutes, and add sulfuric acid to make 25 ml. Stopper the tube, and mix slowly by inverting several times. (Do not shake.)

Standard Solutions Weigh accurately about 0.025 g of propylene glycol, add water to make exactly 100 ml. Place exactly 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml of the resulting solution in five 50-ml volumetric flasks, respectively. To each, add water to make up to the volume. Separately measure exactly 1 ml of these solutions into graduated test tubes, and add dropwise 8 ml of sulfuric acid with the tubes immersed in cold water. Proceed in the same manner as for the test solution, and prepare standard solutions.

Procedure Immediately transfer the test solution into a cell designed for absorbance measurement, and after exactly 5 minutes, measure the absorbance at 580 nm against the reference solution, prepared by treating unmodified starch of the same botanical origin as directed for the test solution. Prepare a calibration curve by measuring the absorbances of the standard solutions in the same manner. Determine the concentration of propylene glycol (μg/mg) from the calibration curve, and calculate the content of hydroxypropyl groups by the following formula.

$$\text{Contents (\%) of hydroxypropyl groups} = \frac{\text{Propylene glycol concentration in the test solution (μg/ml)} \times 0.7763 \times \text{dilution factor}}{\text{Dry-basis weight of the sample} \times 100}$$

(2) Propylene chlorohydrins Not more than 1.0 μg/g.

Test Solution Weigh exactly 30.0 g of Hydroxypropyl Distarch Phosphate into an Erlenmeyer flask, add 125 ml of 1 mol/L of sulfuric acid, and swirl the flask to disperse the contents. Heat in a water bath for 10 minutes, mix the contents well, and heat for an additional 15 minutes. Cool to room temperature, adjust the pH to 7 with sodium hydroxide solution (1 in 4), and filter through glass-fiber filter paper using suction. Collect the filtrate, wash the flask and 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 minutes

until the sodium sulfate is completely dissolved. Transfer the solution into a separating funnel, wash the flask with 25 ml of water, and combine the washings with the sample solution. Extract with five 50-ml portions of diethyl ether, combine the diethyl ether extracts, add 3 g of anhydrous sodium sulfate, and filter through filter paper. Wash the flask and filter paper with 25 ml of diethyl ether, and combine the washings with the filtrate. Evaporate to 4 ml in a 40°C water bath at atmospheric pressure, cool, and add diethyl ether to make exactly 5 ml.

Standard Solutions: Weigh accurately about 0.05 g of propylene chlorohydrin, add water to make exactly 100 ml. Measure exactly 10 ml of this solution, and dilute with water to exactly 100 ml. Use the resulting solution as the standard stock solution. Place 50.0-g portions of unmodified starch of the same botanical origin in five Erlenmeyer flasks, and add 125 ml of 1 mol/L sulfuric acid. To four of the flasks, add exactly 0.5 ml, 1 ml, 2 ml, and 5 ml of the standard stock solution, respectively. No standard stock solution is add to the remaining flask. Then proceed in the same manner for the test solution and prepare the standard solutions.

Procedure: Analyze 1 µl portions of the test solution and the standard solutions by Gas Chromatography using the operating conditions given below. Measure the sum of the peak areas corresponding to 1-chloro-2-propanol and 2-chloro-1-propanol for each standard solution, and prepare a calibration curve for propylene chlorohydrins. Then measure the sum of these two peak areas for the test solution, determine the concentration (µg/ml) of propylene chlorohydrins in the test solution from the calibration curve, and calculate the content of propylene chlorohydrins by the following formula.

$$\text{Content (\%) of propylene chlorohydrins} = \frac{\text{Propylene chlorohydrins concentration in the test solution (µg/ml)} \times 100}{\text{Dry-basis weight of the sample} \times 1{,}000}$$

Operating conditions

Detector: Flame-ionized detector

Detector temperature: 280°C

Column: A silicate glass capillary (15-m length, 0.25-mm internal diameter) coated with polyethylene glycol at 0.25-µm thickness

Column temperature: Maintain at 40°C for 2 minutes, raise to 90°C at 3°C/minute, and maintain for 3 minutes. Thereafter, raise to 250°C at 25°C/minute, and maintain for 5 minutes.

Injection port temperature: 150°C

Injection: Splitless

Carrier gas: Nitrogen or helium

Flow rate: Adjust so that the retention time of 1-chloro-2-propanol is about 13 minutes

(6) Phosphorous Not more than 0.14% as P

Proceed as directed in Purity (4) for Acetylated Distarch Phosphate.

(4) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1)

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (120°C, not more than 13.3 kPa, 4 hours).

Hydroxypropyl Starch

Substance name Hydroxypropyl Starch

CAS number [9049-76-7]

Definition Hydroxypropyl Starch is obtained by etherifying starch with propylene oxide.

Description Hydroxypropyl Starch occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Starch.

Purity (1) Hydroxypropyl groups Not more than 7.0%

Proceed as directed in Purity (3) for Hydroxypropyl Distarch Phosphate.

(2) Propylene chlorohydrins Not more than 1.0 μg/g

Proceed as directed in Purity (2) for Hydroxypropyl Distarch Phosphate.

(3) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(5) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (120°C, not more than 13.3 kPa, 4 hours).

Distarch Phosphate

Substance name Distarch Phosphate

CAS number [55963-33-2]

Definition Distarch Phosphate is obtained by esterifying starch with trimetaphosphate or phosphorus oxychloride.

Description Distarch Phosphate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.
Starch.

Purity (1) Phosphorous Not more than 0.5% as P.
Proceed as directed in Purity (3) for Acetylated Distrach Phosphate.

(2) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (120°C, not more than 13.3 kPa, 4 hours).

Monostarch Phosphate

Substance name Distarch Phosphate

CAS number [63100-01-6]

Definition Distarch Phosphate is obtained by esterifying starch with ortho-phosphoric acid, its sodium or potassium salt, or tripolyphosphate.

Description Distarch Phosphate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (1) for Acetylated Distarch Adipate.

(2) Proceed as directed in Identification (2) for Acetylated Distarch Adipate.

Purity (1) Phosphorous Not more than 0.5% as P.

Proceed as directed in Purity (3) for Acetylated Distrach Phosphate.

(2) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (6) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (120°C, not more than 13.3 kPa, 4 hours).

Phosphated Distarch Phosphate

Substance name Phosphated Distarch Phosphate

Definition Phosphated Distarch Phosphate is obtained through esterification of starch with orthophosphoric acid, its sodium or potassium salt, or sodium tripolyphosphate, combined with esterification with sodium trimetaphosphate or phosphorus oxychloride

Description Phosphated Distarch Phosphate occurs as white to off-white powder, flakes, or granules having a faint odor.

Identification (1) Proceed as directed in Identification (2) for Acetylated Distarch Adipate

(2) Proceed as directed in Identification (3) for Acetylated Distarch Adipate.

Starch.

Purity (1) Phosphorous Not more than 0.5% as P.

Proceed as directed in Purity (3) for Acetylated Distarch Phosphate.

(2) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1)

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Sulfur dioxide Not more than 50 μg/g.

Proceed as directed in Purity (5) for Acetylated Distarch Adipate.

Loss on Drying Not more than 21.0% (120°C, not more than 13.3 kPa, 4 hours).

Reagents and test solutions (TSs)

Reagents

Adipic Acid TS Dissolve 1.90 g of adipic acid in 900 ml of warm water, cool to room temperature, and make up to 1L with water.

Ammonium Molybdate TS for Modified Starch Dissolve 50 g of ammonium molybdate in 900 ml of warm water, cool to room temperature, and add water to make 1L.

BANAS#-brilliant Yellow TS Dissolve 0.10 g of 4,4'-bis(4-amino-1-naphthylazo)-2,2'-stylbensulfonate and 0.020 g of brilliant yellow in 8 ml of 0.1 mol/L sodium hydroxide, add 7 ml of water, and make up to 100 ml with methanol.

4,4'-bis(4-amino-1-naphthylazo)-2,2'-stylbensulfonate $C_{44}H_{32}N_6O_8S_2$ Black granules with a metallic luster.

A solution obtained by dissolving this compound in 0.01 mol/L sodium hydroxide exhibits an absorption maximum at about 510 nm.

MO-Bis-trimethylsilyltrifluoroacetamide $CF_3CO[Si(CH_3)_2]N[Si(CH_3)_3]$ A colorless liquid.

Refractive index n_D^{20}: 1.414–1.415

Specific gravity 0.825–0.836

Boiling point 71–72°C

Brilliant Yellow $C_{36}H_{22}N_4Na_4O_{16}S_6$ A orange-brown powder. Soluble in water.

A solution obtained by dissolving Brilliant Yellow in 0.01 mol/L sodium hydroxide exhibits an absorption maximum at about 492 nm.

Dimedone $C_8H_{12}O_2$ A white to pale yellow crystalline powder.

Melting point 145–149°C

Dimedone TS Dissolve 5 g of dimedone in ethanol (99.5), and make 100 ml. Prepare fresh before use.

Glutaric Acid $HOOC(CH_2)_3COOH$ A white, crystalline powder. Soluble in water.

Melting point 95–99°C

Ninhydrin TS for Modified Starch Dissolve 3.0 g of ninhydrin in 5% sodium hydrogen sulfite solution

o-Nitrobenzaldehyde 2-Nitrobenzaldehyde $O_2NC_6H_4CHO$ Pale yellow crystals or crystalline powder. Soluble in alcohol and in diethyl ether, and slightly soluble in water.

Melting point 42–44°C

Octenyl Succinic Anhydride A mixture of the *cis* and *trans* forms of octenyl succinic anhydride. A colorless or pale yellow liquid.

Content Not less than 96.0% of octenyl succinic anhydride ($C_{12}H_{18}O_3$).

Refractive index n_D^{20}: 1.468–1.470

Specific gravity d_{20}^{20}: 1.025–1.028

Assay Transfer about 1.5 g of Octenyl Succinic Anhydride, accurately weighed, into a 200-ml Erlenmeyer flask with a glass stopper, add exactly 25 ml of 0.5 mol/L methanolic morpholine to dissolve, and allow to stand for 1 hour. Titrate the excess morpholine with 0.5 mol/L methanolic hydrochloric acid. Record the volume of the hydrochloric acid consumed as S (ml). Use BANA#8-brilliant yellow TS as the indicator. The endpoint is when the color of the solution changes from red to blue-purple. Perform a blank test, and record the volume of the hydrochloric acid consumed as B (ml). Obtain the contents by the following formula.

Content (%) of octenylsuccinic anhydride ($C_{12}H_{18}O_3$)

$$= \frac{(B-S)\cdot 0.1051}{\text{Dry-basis weight of the sample}} \times 100$$

Propylene chlorohydrin $CH_3CH(OH)CH_2Cl$ A colorless to pale yellow liquid. Soluble in water, in ethanol, and in diethyl ether.

Content Contains not less than 70% of 1-chloro-2-propanol and about 30% of 2-chloro-1-propanol.

Refractive index n_D^{20}: 1.439–1.441

Specific gravity d_{20}^{20}: 1.111–1.116

Boiling point 126–127°C

Assay Determine the content as directed in operating condition (2) in Gas Chromatographic Assay of Flavoring Agents under the Flavoring Substances Tests.

***p*-Rosaniline–Formaldehyde TS** Dissolve 40 mg of *p*-rosaniline hydrochloride in 20 ml of hydrochloric acid, and add water to make 100 ml. Dissolve 8 g of formalin in water, and make 500 ml. Mix equal volumes of the two solutions. Prepare fresh before use.

***p*-Rosaniline Hydrochloride** $(H_2NC_6H_4)_2C{:}C_6H_4{:}NH{\cdot}HCl$

Melting point 266–270°C

Sodium Azide NaN_3 A white, odorless crystalline powder.

Melting point 275°C It decomposes the melting point or lower.

Sodium Carbonate TS Dissolve 10.6 g of anhydrous sodium carbonate in water, and make 100 ml.

Vanadic acid TS Dissolve 2.5 of Ammonium metavanadate in 600 ml of boiling water, and cool to 60–70°C. Add 20 ml of nitric acid, cool to room temperature, and add water to make 1,000 ml.

Vinyl Acetate $CH_3COOH{=}CH_2$ A colorless, transparent liquid. Soluble in water.

Refractive index n_D^{20}: 1.394–1.396

Specific gravity d_4^{20}: 0.9300

Boiling point 72–73°C

Zinc Acetate TS Dissolve 120 g of zinc acetate dihydrate in 880 ml of water. Before use, filter through quantitative filter (5C).

Volumetric Solution

0.5 mol/L Hydrochloric Acid, Methanolic Contains 18.23 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1,000 ml.

To 45 ml of hydrochloric acid, add 45 ml of water, and made up to 1,000 ml with ethanol. Standardize before use.

Standardization Weigh accurately about 0.6–0.7 g of sodium carbonate (standard reagent), previously dried at 600°C for 1 hour, and dissolve in 20 ml of water. Titrate the resulting solution with the methanolic hydrochloric acid (indicator: 2 drops of bromophenolblue TS). Near the endpoint, boil to remove the carbon oxide, and after cooling, continue the titration. The endpoint is when the color of the solution changes from blue-purple to blue-green.

1 ml of 0.5 mol/L hydrochloric acid = 26.50 mg Na_2CO_3

0.5 mol/L Methanolic Hydrochloric Acid See 0.5 mol/L Hydrochloric Acid, Methanolic

0.5 mol/L Methanolic Morpholine See 0.5 mol/L Morpholine, Methanolic

0.5 mol/L Morpholine, Methanolic Contains 43.56 g of morpholine (C_4H_9NO, molecular weight: 87.12)

Add methanol to 11 ml of morpholine to make 250 ml.

0.45 mol/L Sodium Hydroxide

Contains 18.00 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Using about 20 g of sodium hydroxide, prepare, standardize, and store, as directed for 1 mol/L Sodium Hydroxide. Restandardize frequently.