Amendment to the Ordinance for Enforcement of the Food Sanitation Act and the Specifications and Standards for Foods, Food Additives, Etc.

The government of Japan will designate Psicose Epimerase as an authorized food additive and establish the standards for use and the compositional specifications.

Summary

Japan prohibits the sale etc. of food additives which are not designated by the Minister of Health, Labour and Welfare (hereinafter referred to as "the Minister") under Article 10 of the Food Sanitation Act (Act No. 233 of 1947; hereinafter referred to as "the Act"). In addition, when specifications or standards for food additives are stipulated in the Specifications and Standards for Foods, Food Additives, Etc. (Ministry of Health and Welfare Notification No. 370, 1959) pursuant to Article 11 of the Act, Japan prohibits the sale etc. of those additives unless they meet the specifications or the standards.

In response to a request from the Minister, the Committee on Food Additives of the Food Sanitation Council under the Pharmaceutical Affairs and Food Sanitation Council (hereinafter referred to as "the Committee") has discussed the adequacy of the designation of Psicose Epimerase as a food additive. The conclusion of the Committee is outlined below.

Outline of conclusion

The Minister, pursuant to Article 10 of the Act, should designate Psicose Epimerase as a food additive having no risk to harm human health and establish the standards for use and the compositional specifications pursuant to Article 11 of the Act (see Attachment for the details).

Psicose Epimerase

プシコースエピメラーゼ

Standard for Use (draft)

Not established.

Compositional Specifications (draft)

Substance Name Psicose Epimerase, Allulose Epimerase

CAS Number [1618683-38-7]

Definition Psicose Epimerase is an enzyme that mutually isomerizes fructose and psicose to each other. It is derived from the culture of *Escherichia coli* (limited to *E. coli* K12 W3110 strain) in which the psicose epimerase gene, intrinsically occurring in the bacterium (limited to *Arthrobacter globiformis*), is introduced.

It may contain foods used exclusively for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its activity. It may also contain food additives used for bulking, powdering, diluting, stabilizing, or preserving it or for adjusting its pH or activity.

Enzyme Activity Psicose Epimerase has an enzyme activity of not less than 230 units per gram.

Description Psicose Epimerase occurs as a light to dark brown liquid or as a gray powder.

Identification When tested by the enzyme activity determination specified below, Psicose Epimerase shows activity.

Purity

(1) Lead Not more than 5 µg/g as Pb (0.80 g, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method). If the residue does not dissolve in 5 mL of diluted nitric acid (1 in 100) in the preparation of the test solution specified in Method 1, proceed as directed in Method 3.

(2) Arsenic Not more than 3 µg/g as As (0.50 g, Method 5, Standard Color: Arsenic Standard Solution 3.0 mL, Apparatus B).

Microbial Limits Proceed as directed under Microbial Limit Tests.

Total plate count: Not more than 50,000 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid　Prepare as directed in Method 3 for total plate count.

Pre-enrichment Culture　Prepare as directed in Method 3 for the *Escherichia coli* test and Method 2 for the *Salmonella* test.

Enzyme Activity Determination

(i) Substrate Solution　Weigh 0.18 g of D(+)-psicose, dissolve it in water, and add water to make exactly 5 mL. Prepare fresh before use.

(ii) Sample Solution　Weigh accurately about 1.0 g of Psicose Epimerase, and dissolve it in the diluent prepared as directed below to make a constant volume so that the resulting solution has 4–10 units per mL.　The diluent: Mix phosphate buffer (0.05 mol/L) at pH 8.0 and magnesium chloride TS (1 mol/L) at a rate of 199 : 1.

(iii) D(–)-Fructose Standard Solutions　Weigh accurately about 0.27 g of D(–)-fructose for enzyme activity determination, dissolve it in water to make exactly 100 mL. Use this solution as the standard stock solution. Prepare four standard solutions with different concentrations—10 μmol, 5 μmol, 3 μmol, and 1 μmol of D(–)-fructose ($C_6H_{12}O_6$ = 180.16) per mL—by exactly diluting the standard stock solution with water to 1.5, 3, 5, and 15 times, respectively.

(iv) Procedure

Test Solution　Place 0.100 mL of the sample solution into a test tube, mix it with 0.400 mL of the diluent prepared in (ii), lid the test tube, and equilibrate at 50°C ± 0.5°C for 5 minutes. Add 0.500 mL of the substrate solution to the test tube, mix, incubate at 50°C ± 0.5°C for exactly 10 minutes, and then heat in a water bath for 2 minutes. After cooling, add about 100 mg of strongly acidic cation-exchange resin and about 100 mg of weakly basic anion-exchange resin (free-form), both whose surface water was previously removed with a filter paper, shake for 15 minutes, and filter through a membrane filter (pore size: 0.2 μm).

For strongly acidic cation-exchange resin, wash with water before use as directed in the Strongly Acidic Cation-exchange Resin section under C. REAGENTS, SOLUTIONS, AND OTHER REFERENCE MATERIALS, beginning with "Weigh about 50 g of strongly acidic cation-exchange resin," and confirm that the pH of the effluent is 5.0–6.5.

Reference Solution　Place 0.100 mL of the diluent into a test tube instead of the sample solution, and proceed as directed for the test solution.

Determination　Analyze 10 μL portions of the test solution, the reference solution, and the four D(–)-fructose standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve from the peak area and the concentration (μmol/mL) of each D(–)-fructose standard solution. Measure the peak areas of D(–)-fructose in the test solution and the reference solution, and then determine the concentration (μmol/mL) of D(–)-fructose in each solution from the calibration curve. Calculate the enzyme activity by the following formula. One unit of the enzyme activity

is equivalent to the amount of the enzyme required to liberate 1 μmol of D(−)-fructose per minute when determined as directed in the Procedure.

$$\text{Enzyme activity (unit/g)} = \frac{(C_T - C_B) \times V_T}{M}$$

C_T = concentration (μmol/mL) of D(−)-fructose in the test solution,
C_B = concentration (μmol/mL) of D(−)-fructose in the reference solution,
V_T = whole volume (mL) of the sample solution prepared,
M = weight (g) of the sample.

Operating conditions
Detector: Differential refractometer.
Column: A stainless steel tube (8 mm internal diameter and 30 cm length).
Column packing material: About 9-μm cation-exchange resin for liquid chromatography (Ca-form).
Column temperature: 80°C.
Mobile phase: Water.
Flow rate: 0.4 mL/min.

Reagents, Solutions, and Other Reference Materials

D(−)-Fructose for Enzyme Activity Determination $C_6H_{12}O_6$ [57-48-7] Colorless to white crystals or powder.

Specific rotation $[\alpha]_D^{20}$: −90 to −94° Weigh accurately about 4 g of D(−)-fructose for enzyme activity determination, add 0.2 mL of ammonia TS and 80 mL of water to dissolve it, allow to stand for 30 minutes, and add water to make exactly 100 mL. Measure the optical rotation of the resulting solution.

Purity (1) Clarity of solution Clear (1.0 g, water 20 mL).

(2) Loss of drying Not more than 2.0% (reduced pressure, 18 hours).

(3) Related substances Prepare a test solution by dissolving 20 mg of D(−)-fructose for enzyme activity determination in 2 mL of water. Prepare a control solution by diluting exactly measured 1 mL of the test solution with water to exactly 50 mL. Analyze 10 μL portions of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for three times the retention time of the main peak, and measure the peak areas. The sum of the areas of all peaks, other than the main peak and the solvent peak, from the test solution is not greater than the area of main peak from the control solution.

Operating conditions
Detector: Differential refractometer.

Column: A stainless steel tube (3–8 mm internal diameter and 15–30 cm length).
Column packing material: 5–10 μm aminopropyl-bonded silica gel for liquid chromatography.
Column temperature: A constant temperature of 35–40°C.
Mobile phase: A 7 : 3 mixture of acetonitrile/water.
Flow rate: Adjust the retention time of D(−)-fructose to 4–7 minutes.

Magnesium Chloride TS (1 mol/L) Dissolve 203 g of magnesium chloride hexahydrate in water to make 1000 mL.

D(+)-Psicose $C_6H_{12}O_6$ [551-68-8] A white to slightly pale yellow crystalized powder or powder.

Specific rotation $[\alpha]_D^{20}$: +2.0 to +6.0° (0.1 g, water, 10 mL).

Purity Related substances Prepare a test solution by dissolving 20 mg of D(+)-psicose in 2 mL of water. Prepare a control solution by diluting exactly measured 1 mL of the test solution with water to exactly 50 mL. Analyze 10 μL portions of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for three times the retention time of the main peak, and measure the peak areas. The sum of the areas of all peaks, other than the main peak and the solvent peak, from the test solution is not greater than the area of main peak from the control solution.

Operating conditions
Detector: Differential refractometer.
Column: A stainless steel tube (3–8 mm internal diameter and 15–30 cm length).
Column packing material: 5–10 μm aminopropyl-bonded silica gel for liquid chromatography.
Column temperature: A constant temperature of 35–40°C.
Mobile phase: A 7 : 3 mixture of acetonitrile/water.
Flow rate: Adjust the retention time of D(+)-psicose to 6–9 minutes.