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 chitin-glucan as an authorized food additive and establish compositional specifications and use standards for this additive.

Background

Japan prohibits the sale of food additives that are not designated by the Minister of Health, Labour and Welfare (hereinafter referred to as "the Minister") under Article 12 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. (Public Notice of the Ministry of Health and Welfare No. 370, 1959), Japan prohibits the sale of those additives unless they meet the specifications or the standards pursuant to Article 13 of the Act.

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 chitin-glucan as a food additive. The conclusion of the Committee is outlined below.

Outline of conclusion

The Minister should designate chitin-glucan as a food additive unlikely to cause harm to human health pursuant to Article 12 of the Act and should establish compositional specifications and use standards for this additive pursuant to Article 13 of the Act (see Attachment for the details).

Attachment

Chitin-Glucan

キチングルカン

Standards for Use (draft)

Permitted for use in grape juice used for wine production and grape wine only.

Shall be used at not more than 5 g/L in grape wine as chitin-glucan. When Chitin-Glucan is used in grape juice for wine production, this additive is considered to be used in grape wine.
The Chitin-Glucan used shall be removed before the completion of the final product.

Compositional Specifications (draft)

Substance Name Chitin-Glucan

Definition
Chitin-Glucan is a copolymer composed of chitin and β-1,3-glucan that are derived from the culture of filamentous fungi (limited to *Aspergillus niger*).

Content
Chitin-Glucan contains not less than 95% of chitin-glucan.

Description Chitin-Glucan occurs as a white to light yellow-brown powder. It is odorless.

Identification
Chitin/glucan ratio 25：75 to 60：40.

Sample Place 2.0 g of Chitin-Glucan in a centrifuge tube, and add 40 mL of hydrochloric acid TS (1 mol/L). After shaking it for 30 minutes, centrifuge at 3000 rpm for 10 minutes, and remove the supernatant. Again add 40 mL of hydrochloric acid TS (1 mol/L), and perform the same step. Then, add 40 mL of water to the residue, agitate, centrifuge at 3000 rpm for 10 minutes, and remove the supernatant. Repeat the same step with 40 mL of water at each time until the electric conductivity of the supernatant is below 100 μS/cm. Then, add 40 mL of ethanol (99.5) to the residue, agitate, centrifuge at 3000 rpm for 10 minutes, and remove the supernatant. Again add 40 mL of ethanol (99.5) to the residue, and perform the same step. Then, add 40 mL of a 1：1 mixture of chloroform/methanol to the residue, shake for 30 minutes, centrifuge at 3000 rpm for 10 minutes, and remove the supernatant. Again

add 40 mL of a 1 : 1 mixture of chloroform/methanol to the residue, and perform the same step. Then, add 40 mL of acetone to the residue, shake for 30 minutes, and centrifuge at 3000 rpm for 10 minutes. Filter the supernatant through a filter paper (30 µm pore size), and discard the filtrate. Again add acetone to the residue in the centrifuge tube, shake, filter the whole content in the tube through the filter paper, and remove the filtrate. Place the residue with the filter paper on a watch glass, and dry it at room temperature in a draft chamber. Use the resulting residue on the filter paper as the sample.

Procedure Put the sample in a solid-state NMR tube (3–4 mm external diameter), and seal tightly. Measure the CP/MAS ^{13}C NMR spectrum using an NMR spectrometer— conditioned so that the carbon signal from adamantane in the high magnetic field is δ 29.5 ppm—with a proton resonance frequency of 400 MHz or more under the following operating conditions. Separately, measure the CP/MAS ^{13}C NMR spectrum of chitin as directed for the sample. For the obtained spectra, correct the baseline and conduct waveform separation treatment. Confirm that signals are detected in each CP/MAS ^{13}C NMR spectrum of the sample and chitin at SN ratio 50 or more in the regions of around δ 23 ppm, δ 55 ppm, δ 61 ppm, and δ104 ppm. Designate each signal area intensity as A_1, A_2, A_3, and A_4 for the sample; and B_1, B_2, B_3, and B_4 for chitin, respectively. Determine the chitin composition percentage (%) and the glucan composition percentage (%) by the following formulae. The chitin/glucan ratio is expressed as the ratio of chitin composition percentage (%) and the glucan composition percentage (%).

$$\text{Chitin composition ratio (\%)} = \frac{C_1 + C_2 + C_3 + C_4}{4} \times 100$$

$$\text{Glucan composition ratio (\%)} = 100 - \text{Chitin composition ratio (\%)}$$

A_1 = signal area intensity of Chitin-Glucan at around δ 23 ppm,
A_2 = signal area intensity of Chitin-Glucan at around δ 55 ppm,
A_3 = signal area intensity of Chitin-Glucan at around δ 61 ppm,
A_4 = signal area intensity of Chitin-Glucan at around δ 104 ppm,
B_1 = signal area intensity of Chitin at around δ 23 ppm,
B_2 = signal area intensity of Chitin at around δ 55 ppm,
B_3 = signal area intensity of Chitin-Glucan at around δ 61 ppm,
B_4 = signal area intensity of Chitin-Glucan at around δ 104 ppm,
$C_1 = (B_3/B_1)/(A_3/A_1)$,
$C_2 = (B_3/B_2)/(A_3/A_2)$,
$C_3 = (B_4/B_1)/(A_4/A_1)$,

$C_4 = (B_4/B_2)/(A_4/A_2)$.

Operating conditions

Spinning rate: Not less than 7 kHz.

Contact time: Constant time at around 2 milliseconds.

Delay time: Not less than 5 seconds.

Number of accumulation: Not less than 3000.

Purity

(1) Lead Not more than1 µg/g as Pb (an amount equivalent to 4.0 g on the dried basis, Method 1, Control Solution: Lead Standard Solution 4.0 mL, Flame Method).

(2) Arsenic Not more than 1 µg/g as As (an amount equivalent to 1.0 g on the dried basis, Method 3, Standard Color: Arsenic Standard Solution 2.0 mL, Apparatus B).

Loss on Drying Not more than 10% (105°C, 3 hours).

Ash Not more than 3% (600°C, 6 hours, on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests.

Total plate count: Not more than 1000 per gram.

Yeasts and molds: Not more than 200 per gram.

Escherichia coli: Negative per test.

Salmonella: Negative per test.

Sample Fluid Prepare as directed in Method 1 for the total plate count and the enumeration of yeasts and molds.

Pre-enrichment Culture Prepare as directed in Method 1 for the *Escherichia coli* test and the *Salmonella* test.

Assay Weigh accurately about 5 g of Chitin-Glucan, transfer it into a flask, add 100 mL of water, and stir for 2 minutes. Filter the resulting suspension by suction through a membrane filter (1 µm pore size). Place the filtrate in an evaporating dish, previously dried at 105°C for 30 minutes, cooled in a desiccator, and accurately weighed (the mass of the dish = m g), and evaporate it to dryness. Then, dry it at 105°C for 4 hours, leave to cool in the desiccator, accurately weigh the mass (M g), and calculate the content.

$$\text{Content (\%) of chitin-glucan} = \frac{\text{Weight (g) of the sample} - (\text{M (g)} - \text{m (g)})}{\text{Weight (g) of the sample}} \times 100$$

Reagents, Solutions, and Other Reference Materials

Adamantane $C_{10}H_{16}$ [281-23-2] White to light brown crystals or powder.

Purity Related substances Prepare a test solution by dissolving 0.5 g of adamantine in 10 mL of toluene. Prepare a control solution by diluting exactly measured 1.5 mL of the test solution with toluene to exactly 50 mL. Analyze 1.0 μL-portions of the test solution and the control solution by gas chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The sum of the areas of all peaks, excluding the main peak and solvent peaks, from the test solution is not greater than the area of main peak from the control solution.

Operating conditions
Detector: Frame-ionization detector.
Column: A fused silica tube (0.53 mm internal diameter and 15–30 m length) coated with a 5.0 μm thick layer of dimethylpolysiloxane for gas chromatography.
Column temperature: Raise the temperature at 10°C/minute from 100°C to 250°C and maintain the temperature at 250°C for 5 minutes.
Injection port temperature: 250°C.
Detector temperature: 250°C.
Carrier gas: Helium.
Flow rate: Adjust so that the peak of adamantine appears about 6–12 minutes after injection.
Injection method: Split.
Split ratio: 1 : 20.

Chitin $(C_8H_{13}NO_5)_n$ [1398-61-4] A white to light brown powder or scale-like substance.

Identification Add 1 g of chitin to 200 mL of diluted acetic acid (1 in 100). It does not solve.

Loss on drying Not more than 15.0% (1g, 105°C, 2 hours).