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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.157-2016

**National Food Safety Standard -
Determination of Organic Acid in Foods**

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People's Republic of China**

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Foreword

This Standard is drafted as a replacement of GB/T 5009.157-2003 "Determination of Organic Acid in Foods".

In comparison with GB/T 5009.157-2003 "Determination of Organic Acid in Foods", there are several main modifications as follows:

- The name of this Standard is modified into "National Food Safety Standard - Determination of Organic Acid in Foods";
- The scope of application is extended to jelly, solid beverage and canned fruit, etc.;
- Certain tested objects are added to this Standard, such as lactic acid, fumaric acid and adipic acid, etc.

National Food Safety Standard -

Determination of Organic Acid in Foods

1 Scope

This Standard specifies the method of determining tartaric acid, lactic acid, malic acid, citric acid, succinic acid, fumaric acid and adipic acid in foods.

This Standard is applicable to the determination of seven types of organic acid in fruit juice, fruit juice beverage, carbonated beverage, solid beverage, gum-based candy, cookies, pastry, jelly, canned fruit, fresh dough products and fillings in baked goods.

2 Principle

Directly add water to dilute the sample or adopt water to extract the sample; adopt strong anion exchange solid phase extraction column for purification; adopt reversed phase column for separation. Determine the nature through the retention time; quantify with the external standard method.

3 Reagents and Materials

Unless otherwise indicated, the reagents adopted under this method are of analytical purity. The water is first-grade water as specified in GB/T 6682.

3.1 Reagents

3.1.1 Methanol (CH_3OH): chromatographic purity.

3.1.2 Anhydrous ethanol ($\text{CH}_3\text{CH}_2\text{OH}$): chromatographic purity.

3.1.3 Phosphoric acid (H_3PO_4).

3.2 Preparation of Reagents

3.2.1 Phosphoric acid solution (0.1%): weigh-take 0.1 mL of phosphoric acid; add water to dilute to 100 mL; mix it up.

3.2.2 Phosphoric acid - methanol solution (2 %): weigh-take 2 mL of phosphoric acid; add methanol to dilute to 100 mL; mix it up.

3.3 Standards

5.2 Sample Processing

5.2.1 Fruit juice beverage, fruit juice and fruity carbonated beverage

Weigh-take 5 g (accurate to 0.01 g) of homogenized sample (heat up the sample to remove carbon dioxide if the sample contains carbon dioxide), place it in 25 mL volumetric flask; add water to the constant volume; adopt 0.45 μ m aqueous membrane to filter the sample; inject it into high-performance liquid chromatograph for analysis.

5.2.2 Jelly and canned fruit

Weigh-take 10 g (accurate to 0.01 g) of homogenized sample, place it in 50 mL plastic centrifuge tube, then, add 20 mL of water; homogenize and extract at 15,000 r/min for 2 min, start centrifugation at 4,000 r/min for 5 min; take the upper extract and place it in 50 mL volumetric flask. Use 20 mL of water to re-extract the remains; combine the extract in the same volumetric flask. Use water to dilute to the constant volume; adopt 0.45 μ m aqueous membrane to filter the sample; inject it into high-performance liquid chromatograph for analysis.

5.2.3 Gum-based candy

Weigh-take 1 g (accurate to 0.01 g) of homogenized sample, place it in 50 mL plastic centrifuge tube which is equipped with a plug; add 20 mL of water, then, start oscillation extraction on a swirling mixer for 5 min; start centrifugation for 3 min at 4,000 r/min, then, transfer the supernatant into 100 mL volumetric flask; add 20 mL of water to re-extract the remains; combine the extract in the sample volumetric flask. Use anhydrous ethanol (3.1.2) to dilute to the constant volume, shake it well.

Accurately remove-take 10 mL of supernatant and place it in 100 mL heart-shaped bottle; add 10 mL of anhydrous ethanol (3.1.2) to the bottle, then, rotate and concentrate it under 80°C $\pm$ 2°C till it approaches dryness; add 5 mL of anhydrous ethanol (3.1.2) to continue the concentration, till it reaches absolute dryness. Use 1 mL x 1 mL of water to rinse the heart-shaped bottle twice. Transfer all the liquid to be purified to solid phase extraction column (SAX) that's pre-activated, control the flow rate at 1 mL/min~2 mL/min, then, remove the effluent. Use 5 mL of water to rinse the purification column; use 5 mL of phosphoric acid - methanol solution (3.2.2) to elute it, control the flow rate at 1 mL/min~2 mL/min; gather the eluent and place it into 50 mL heart-shaped bottle; rotate the eluent under 45 °C and evaporate till it approaches dryness; add 5 mL of anhydrous ethanol (3.1.2) to continue the concentration, till it reaches absolute dryness. Use 1.0 mL of phosphoric acid solution (3.2.1) to oscillate and dissolve the remains; adopt 0.45 μ m membrane to filter it; inject it into high-performance liquid chromatograph for analysis.

5.2.4 Solid beverage

Weigh-take 5 g (accurate to 0.01 g) of homogenized sample, place it in 50 mL beaker. Add 40 mL of water to dissolve it, and transfer it into 100 mL volumetric flask. Adopt anhydrous ethanol (3.1.2) to dilute to the constant volume; shake it well, place it evenly for 10 min.

Accurately remove-take 20 mL of supernatant and place it in 100 mL heart-shaped

the balance for 5 min.

5.3.1.3 Column temperature: 40 °C.

5.3.1.4 Inlet volume: 20 µL.

5.3.1.5 Detection wavelength: 210 nm.

5.3.2 Determination of adipic acid

5.3.2.1 Chromatographic column: CAPECELL PAK MG S5 C₁₈ column, 4.6 mm x 250 mm, 5 µm, or other chromatographic columns with equivalent performance.

5.3.2.2 Mobile phase: use 0.1% phosphoric acid solution - methanol=75+25 (volume ratio) equivalent flow to elute for 10 min.

5.3.2.3 Column temperature: 40 °C.

5.3.2.4 Inlet volume: 20 µL.

5.3.2.5 Detection wavelength: 210 nm.

5.4 Draw a Standard Curve Line

Respectively inject standard series of working fluid into high-performance liquid chromatograph; determine the corresponding peak height or peak area. Take the concentration of standard working fluid as x-coordinate, take the peak height or peak area as y-coordinate, and draw a standard curve line (please refer to Appendix A for the standard curve line).

5.5 Determination of Sample Solution

Inject the sample solution into high-performance liquid chromatograph, obtain the peak height or peak area. Obtain the concentration of organic acid in the test solution in accordance with the standard curve line.

6 Expression of Analysis Results

The content of organic acid in the sample shall be calculated in accordance with Formula (1):

$$X = \frac{C \times V \times 1\,000}{m \times 1\,000 \times 1\,000} \dots\dots\dots (1)$$

Where:

X - The content of organic acid in the sample, expressed in (g/kg);

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