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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.149-2016

**National Food Safety Standard -
Determination of Gardenia Yellow in Foods**

食品安全国家标准

食品中栀子黄的测定

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State Food and Drug Administration.**

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National Food Safety Standard -

Determination of Gardenia Yellow in Foods

1 Scope

This Standard specifies the determination method of crocin and crocetin, the representative components of gardenia yellow in food.

This Standard applies to the determination of crocin and crocetin in ice cream, candied fruit, pickle, dried almond, chocolate, pastries, cooked meat, soy sauce, fruit juice, mixed wine, jelly and potato chip.

2 Principle

The sample which is ultrasonically extracted by methanol, shall be separated by C₁₈ reversed-phase chromatography / visible light detector to determine crocin and crocetin, major color-developing components of gardenia yellow, at 440 nm. It is characterized by retention time and quantified by external standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-1 water which is specified by GB/T 6682.

3.1 Reagents

3.1.1 Methanol (CH₃OH): chromatographic pure.

3.1.2 Acetonitrile (CH₃CN): chromatographic pure.

3.1.3 Glacial acetic acid (CH₃COOH).

3.1.4 Ammonium acetate (CH₃COONH₄).

3.2 Preparation of reagents

3.2.1 Acetic acid-ammonium acetate solution (pH=4): accurately weigh 0.77 g of ammonium acetate in a 1 L volumetric flask; add 900 mL of water to dissolve; use glacial acetic acid to adjust pH=4.0; add water to fix volume to 1 L; mix well. Use it after it is filtered through a 0.45 μm microporous membrane.

5.1.1 Liquid sample

Shake well and pack the samples such as beverage, alcohol and soy sauce; seal and store at room temperature or in a refrigerator.

5.1.2 Semi-solid sample

For samples such as jelly, take the edible portion to homogenate; stir evenly; package; seal; refrigerate or freeze to store.

5.1.3 Solid sample

For samples of low-water content, such as biscuit, cake, cooked meat products and cocoa products, use the high-speed pulverizer to pulverize; package; seal and store at room temperature out of the sun.

5.2 Sample processing

5.2.1 Liquid sample

Weigh 2 g (accurate to 0.01 g) of uniform sample (carbon dioxide in the sample, if any, shall be removed by ultrasound firstly) in a beaker; use an appropriate amount of methanol to dissolve it; transfer it to a 25 mL volumetric flask; add methanol to fix volume; shake well. Pipette 1 mL of the solution; filter it through a 0.45 µm organic filter for testing.

5.2.2 Semi-solid and solid samples

Weigh 2 g (accurate to 0.01 g) of uniform sample in a 50 mL centrifuge tube; accurately measure 25 mL of methanol solution into it; use ultrasound for 20 min and vortex for 2 min; centrifuge at 4 000 r/min for 10 min; absorb 1 mL of supernatant to filter through a 0.45 µm organic filter membrane, which is for later test.

5.2.3 Soy sauce

Weigh 1 g (accurate to 0.01 g) of uniform sample in a beaker; use an appropriate amount of methanol to dissolve it; transfer it to a 50 mL volumetric flask; add methanol to fix volume; shake well. Pipette 1 mL of the solution; filter it through a 0.45 µm organic filter for testing.

Note: 5.2 shall be performed in an environment without strong light exposure.

5.3 Apparatus reference conditions

Apparatus reference conditions are listed as below:

- a) Chromatographic column: C₁₈ column with a column length of 250 mm, an inner diameter of 4.6 mm, a particle size of 5 µm, or columns of equivalent performance;

X -- the content of crocin or crocetin in the sample, in grams per kilogram (g/kg);

ρ -- the concentration of crocin or crocetin in the sample solution that is obtained from the standard curve, in micrograms per milliliter ($\mu\text{g/mL}$);

V -- the volumetric volume of the sample solution, in milliliters (mL);

1 000 -- conversion coefficient;

m -- the sample mass of the final sample, in grams (g);

F -- the purity conversion factor of the standard.

The calculation result shall keep two significant figures.

7 Precision

The absolute difference of two independent test results under repeatability cannot exceed 15% of the arithmetic mean value.

8 Others

Method detection-limit (LOD): for soy sauce sample, when the sampling amount is 1.0 g, the crocin is 50 mg/kg, and the crocetin is 10.0 mg/kg; for other samples, when the sampling amount is 2.0 g, the crocin is 12.5 mg/kg, the crocetin is 2.5 mg/kg.

Method quantitation-limit (LOQ): for soy sauce sample, when the sampling amount is 1.0 g, the crocin is 250 mg/kg, and the crocetin is 50.0 mg/kg; for other samples, when the sampling amount is 2.0 g, the crocin is 62.5 mg/kg, the crocetin is 12.5 mg/kg.

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