



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

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National food safety standard - Food additives - Mulberry red

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Table of Contents

1 Scope	3
2 Molecular Formula and Structural Formula	3
3 Technical Requirements	3
Appendix A Inspection Method	5

1 Scope

This Standard is applicable to the food additive mulberry red, which is obtained through extraction and refining with mulberry (*Fructus Mori*) fruit and its pomace as the raw materials, and with an aqueous solution containing citric acid and edible ethanol.

2.1 Molecular Formula

Cyanidin-3-glucoside: $[C_{21}H_{21}O_{12}]^+ X^-$ Cyanidin-3-rutinoside: $[C_{27}H_{31}O_{15}]^+ X^-$ X^- is the counter ion.

2.2 Structural Formula

Cyanidin-3-glucoside: R1 = glucosyl Cyanidin-3-rutinoside: R1 = rutinosyl

3.1 Sensory Requirements

The sensory requirements shall comply with the requirements of Table 1.

Appendix A Inspection Method

WARNING: some reagents used in the inspection method of this Standard are toxic or corrosive. During operation, appropriate safety and protective measures shall be taken.

A.1 General Rules When other requirements are not specified, the reagents and water used in this Standard refer to analytically pure reagents and Grade-3 water specified in GB/T 6682.

When other requirements are not specified, the standard solutions, preparations and products used in tests shall be prepared in accordance with the stipulations of GB/T 601, GB/T 602 and GB/T 603. When it is not specified which solvent is used for preparation, the solutions used in tests refer to aqueous solutions.

A.2 Identification Test
A.2.1 Reagents and materials
A.2.1.1 Trifluoroacetic acid: chromatographically pure.

A.2.1.2 Acetonitrile: chromatographically pure.

A.2.1.3 Sodium hydroxide solution: 2 mol/L.

A.2.1.4 Hydrochloric acid solution: 2 mol/L.

A.2.1.5 Disodium hydrogen phosphate solution: 0.2 mol/L. Accurately weigh-take

71.64 g of disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$); use water to dissolve it and dilute to a constant volume of 1,000 mL.

A.2.1.6 Citric acid solution: 0.1 mol/L. Accurately weigh-take 21.01 g of citric acid ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$); use water to dissolve it and dilute to a constant volume of 1,000 mL.

A.2.1.7 Citric acid - disodium hydrogen phosphate buffer solution: pH 3.0. Measure-take 41 mL of 0.2 mol/L disodium hydrogen phosphate solution and 159 mL of 0.1 mol/L citric acid solution; mix them up.

A.2.1.8 Trifluoroacetic acid solution: 0.1% (volume fraction).

A.2.1.9 Extraction solvent: trifluoroacetic acid solution + acetonitrile (75 + 25).

A.2.1.10 Cyanidin-3-glucoside (kuromanin chloride) reference substance: CAS: 7084-24-4, purity $\geq 98\%$.

volume of 100 mL; filter them through a 0.45 μm filter membrane.

A.2.3.3.3 Reference chromatographic conditions A.2.3.3.3.1 Chromatographic column: C18 reversed phase column (3 mm $\times$ 150 mm, 3.5 μm), or other equivalent chromatographic columns.

A.2.3.3.3.2 Mobile phase A: trifluoroacetic acid solution (A.2.1.8).

A.2.3.3.3.3 Mobile phase B: acetonitrile (A.2.1.2).

A.2.3.3.3.4 Flow rate: 0.4 mL/min.

A.2.3.3.3.5 Detection wavelength: 520 nm.

A.2.3.3.3.6 Injection volume: 2 μL .

A.2.3.3.3.7 Column temperature: 35 $^{\circ}\text{C}$.

A.2.3.3.3.8 Isocratic elution conditions: mobile phase A + mobile phase B = 89 + 11.

A.2.4 Analytical procedures Under the reference chromatographic conditions of A.2.3.3.3, respectively inject the reference solution and specimen solution of cyanidin-3-glucoside (kuromanin chloride)

and cyanidin-3-rutinoside.

A.2.5 Result determination The chromatogram of the specimen solution shall manifest two obvious main peaks, and the retention time of the two main peaks shall be consistent with

the retention time of the reference substance of cyanidin-3-glucoside (kuromanin chloride) and cyanidin-3-rutinoside.

A.3 Determination of Color Value A.3.1 Reagent and material Citric acid - disodium hydrogen phosphate buffer solution: pH 3.0. Same as A.2.1.7.

A.3.2 Instruments and equipment A.3.2.1 UV spectrophotometer.

A.3.2.2 Cuvette: 1 cm.

A.3.3 Analytical procedures Inject the specimen solution (A.2.3.2) into a 1 cm cuvette. Take the citric acid - disodium hydrogen phosphate buffer solution (A.2.1.7) as the blank; use the spectrophotometer the distillation method in GB 5009.34, expressed in (g/kg);

1,000---the unit conversion factor.

---the color value of the specimen being tested.

The arithmetic mean value of parallel determination results shall prevail in the test result. The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 10% of the arithmetic mean value.

A.5 pH Determination A.5.1 Instrument and equipment pH meter.

A.5.2 Determination method Weigh-take 1 g of specimen, accurate to 0.01 g. Completely dissolve it in distilled water and dilute to a constant volume of 100 mL. Use the pH meter to determine its pH value.

The arithmetic mean value of parallel determination results shall prevail in the test result. The absolute difference between two independent determination results obtained under repeatability conditions shall not exceed 0.1 pH.

A.6 Determination of Burning Residue A.6.1 Instrument and equipment A.6.1.1 Crucible.

A.6.1.2 High-temperature furnace.

A.6.1.3 Desiccator.

A.6.2 Determination method Weigh-take 3 g of specimen, accurate to 0.001 g. Place it in a crucible that has reached a constant mass at 800 °C +/- 25 °C. Firstly, slowly carbonize it on an electric furnace (at about 300 °C), then, transfer it into a high-temperature furnace at 800 °C +/- 25 °C to burn to a constant mass.

A.6.3 Result calculation The mass fraction w_1 of the burning residue shall be calculated in accordance with Formula (A.3).