NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 1886.311-2020

National food safety standard - Food additives - Black Currant Red

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1 Scope

This Standard is applicable to the food additive of black currant red made from Ribes nigrum L fruit or pomace, and through the extraction and refining by water or (and)

edible ethanol, and other processes.

2.1 Molecular formula

Cyanidin 3-rutinoside: [C27H31O15]+X-Delphinidin 3-rutinoside: [C27H31O16]+X-Cyanidin 3-glucoside: [C21H21O11]+X-Delphinidin 3-glucoside: [C21H21O12]+X-X- is a counter ion.

2.2 Structural formula

Cyanidin 3-rutinoside: R1 = H, R2 = rutinoside;

Delphinidin 3-rutinoside: R1 = OH, R2 = rutinoside;

Cyanidin 3-glucoside: R1 = H, R2 = glucoside;

Delphinidin 3-glucoside: R1 = OH, R2 = glucoside.

Appendix A Test Methods

A.1 Safety tips Some reagents used in the test methods of this Standard are toxic or corrosive; and appropriate safety and protective measures shall be taken during operation.

A.2 General rules The reagents and water used in this Standard refer to analytically-pure reagents and Class-III water specified in GB/T 6682 when other requirements are not indicated. The standard solutions, formulations and products used in the test are prepared in accordance with the provisions of GB/T 601, GB/T 602, and GB/T 603 when no other requirements are specified. The solution used in the test refers to an aqueous solution when it is not specified which solvent is used for preparation.

A.3 Identification test A.3.1 Reagents and materials A.3.1.1 Sodium hydroxide solution: 43.0g/L.

A.3.1.2 Disodium hydrogen phosphate solution: 0.2mol/L. Accurately take 71.64g of disodium hydrogen phosphate (Na2HPO4.12H2O); dissolve and make constant volume by water to 1000mL.

A.3.1.3 Citric acid solution: 0.1mol/L. Accurately take 21.01g of citric acid (C6 H8O7 .H2O); dissolve and make constant volume to 1000mL.

A.3.1.4 Citric acid-disodium hydrogen phosphate buffer solution: pH 3.0. Mix 41mL of 0.2mol/L disodium hydrogen phosphate solution and 159mL of 0.1mol/L citric acid solution.

A.3.1.5 Hydrochloric acid solution: 0.3% (volume fraction).

A.3.1.6 Ethyl acetate.

A.3.1.7 Cyanidin 3-rutinoside reference substance: purity >=98%.

A.3.1.8 Delphinidin 3-rutinoside reference substance: purity >=98%.

A.3.2 Apparatus A.3.2.1 Ultraviolet spectrophotometer.

A.3.3.3.3.4 Column temperature: 35°C.

A.3.3.3.3.5 Flow rate: 1.0mL/min.

A.3.3.3.3.6 Detection wavelength: 520nm.

A.3.3.3.3.7 Injection volume: 10uL.

A.3.3.3.3.8 Elution conditions: isocratic elution, mobile phase A + mobile phase B = 20 + 80.

A.3.3.3.4 Analysis procedures Under A.3.3.3.3 reference chromatographic conditions, cyanidin 3-rutinoside and delphinidin 3-rutinoside reference solution and sample solution were injected once, respectively.

A.3.3.3.5 Judgment of results The retention time of the two main peaks in the chromatogram of the specimen solution shall be consistent with the retention time of the cyanidin 3-rutinoside and delphinidin 3-rutinoside reference substance.

A.4 Determination of color scale A.4.1 Reagents and materials Citric acid-disodium hydrogen phosphate buffer solution: pH 3.0. The same as A.3.1.4.

A.4.2 Apparatus A.4.2.1 UV spectrophotometer.

A.4.2.2 Cuvette: 1cm.

A.4.3 Analysis procedures Inject the specimen solution (A.3.3.2) into a 1cm cuvette; use the citric acid-disodium hydrogen phosphate buffer solution (A.3.1.4) as a blank; and use a spectrophotometer to measure the absorbance value (the absorbance value shall be controlled between

0.2 and 0.7; otherwise, the concentration of the specimen solution shall be adjusted, and then the absorbance shall be measured again) at the maximum absorption wavelength in the range of 510nm~520nm.

A.4.4 Calculation of results The color scale is calculated as the absorbance E10% 1cm (510~520) nm measured at the maximum absorption wavelength in the range of 510nm~520nm by the specimen A.6 Determination of basic pigment A.6.1 Reagents and materials A.6.1.1 Ether.

A.6.1.2 Sodium hydroxide solution: 10g/L.

A.6.1.3 Acetic acid solution: take 60mL of glacial acetic acid; dilute and make constant volume by water to 1000mL.

A.6.2 Analysis procedures Take 1g of specimen (accurate to 0.01g); add sodium hydroxide solution (A.6.1.2) to dissolve and make constant volume to 100mL; shake well. Then take 30mL of solution;

add 15mL of ether for extraction; leave ether after layering; then extract twice with 5mL of acetic acid solution (A.6.1.3).

A.6.3 Observation and evaluation results If the color of the acetic acid extraction liquid is colorless, then the test is passed.

A.7 Determination of other acid pigments A.7.1 Reagents and materials A.7.1.1 Ammonia solution: take 400 mL of ammonia (28%); add water and make constant volume to 1000mL.

A.7.1.2 Pyridine.

A.7.1.3 Hydrochloric acid.

A.7.1.4 Developing agent: pyridine + ammonia solution (2+1).

A.7.1.5 Stannous chloride solution: take 4g of stannous chloride ($SnCl_2.2H_2O$); place it in a dry beaker; add 40 mL of hydrochloric acid to dissolve and add water to dilute to 100mL.

A.7.2 Analysis procedures Take 1 g of the specimen (accurate to 0.01 g); add 1 mL of ammonia solution (A.7.1.1)

and 10 mL of water to dissolve; shake well. Take the thin-layer plate; accurately perform point sample of 2uL on the starting line 2 cm from the bottom edge; and evaporate the solvent. Use a developing agent to expand with the upward method.

When the solvent front is expanded to 15cm from the starting line, take out the thin layer plate and evaporate the solvent.