GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 1886.355-2022

National food safety standard - Food additive - Stevia

食品安全国家标准 食品添加剂 甜菊糖苷

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State Administration for Market Regulation.

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National food safety standard - Food additive - Calcium Stevia

1 Scope

This standard applies to the food additive stevia, which is obtained by extracting and refining the leaves of Stevia Rebaudiana Bertoni as raw materials. The known glycosides include stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside E, rebaudioside F, rebaudioside M, rebaudioside N, rebaudioside O, dulcoside A, rubusoside, steviolbioside.

2 Molecular formula, structural formula, relative molecular mass

2.1 Molecular formulas of 13 glycosides

Stevioside: $C_{38}H_{60}O_{18}$

Rebaudioside A: $C_{44}H_{70}O_{23}$

Rebaudioside B: $C_{38}H_{60}O_{18}$

Rebaudioside C: $C_{44}H_{70}O_{22}$

Rebaudioside D: $C_{50}H_{80}O_{28}$

Rebaudioside E: $C_{44}H_{70}O_{23}$

Rebaudioside F: $C_{43}H_{68}O_{22}$

Rebaudioside M: $C_{56}H_{90}O_{33}$

Rebaudioside N: $C_{56}H_{90}O_{32}$

Rebaudioside Q: $C_{62}H_{100}O_{37}$

Dulcoside A: $C_{38}H_{60}O_{17}$

Rubusoside: $C_{32}H_{50}O_{13}$

Steviolbioside: $C_{32}H_{50}O_{13}$

2.3 Relative molecular mass of 13 glycosides

Stevioside = 804.87 (according to 2018 International Relative Atomic Mass)

Rebaudioside A: 967.01 (according to 2018 international relative atomic mass)

Rebaudioside B: 804.87 (according to 2018 international relative atomic mass)

Rebaudioside C: 951.01 (according to 2018 international relative atomic mass)

Rebaudioside D: 1129.15 (according to 2018 international relative atomic mass)

Rebaudioside E: 967.01 (according to 2018 international relative atomic mass)

Rebaudioside F: 936.99 (according to 2018 international relative atomic mass)

Rebaudioside M: 1291.29 (according to 2018 international relative atomic mass)

Rebaudioside N: 1275.29 (according to 2018 international relative atomic mass)

Rebaudioside O: 1437.44 (according to 2018 international relative atomic mass)

Dulcoside A: 788.87 (according to 2018 international relative atomic mass)

Rubusoside = 642.73 (according to 2018 international relative atomic mass)

Steviolbioside = 642.73 (according to 2018 international relative atomic mass)

3 Technical requirements

3.1 Sensory requirements

Sensory requirements shall meet the requirements of Table 2.

3.2 Physical-chemical indicators

Physical-chemical indicators shall meet the requirements of Table 3.

Appendix A

Testing method

A.1 General provisions

The reagents and water, which are used in this standard, refer to analytically pure reagents and grade-3 water, which are specified in GB/T 6682, unless otherwise specified. The standard titration solution, standard solution for impurity determination, preparations, products, which are used in the test, shall be prepared in accordance with the provisions of GB/T 601, GB/T 602, GB/T 603, unless otherwise specified. The solution, which is used in the test, refers to the aqueous solution, when the solvent is not specified.

A.2 Identification test

A.2.1 Chromatographic test of stevia

In the stevia content test, the chromatographic peaks of the 13 glycosides, in the chromatogram of the sample solution, shall correspond to the mixed standard solution.

A.3 Determination of stevia content (on a dry basis)

A.3.1 Reagents and materials

A.3.1.1 Acetonitrile: Chromatographically pure.

A.3.1.2 Sodium dihydrogen phosphate: Chromatographically pure.

A.3.1.3 Phosphoric acid: Chromatographically pure.

A.3.1.4 Water: Grade-1 water specified in GB/T 6682.

A.3.1.5 Aqueous solution of acetonitrile: The volume ratio of acetonitrile and water is 30:70.

A.3.1.6 Sodium phosphate buffer (pH 2.6): Weigh 1.20 g of sodium dihydrogen phosphate (NaH_2PO_4); dissolve it in 800 mL of water; use phosphoric acid to adjust the pH to 2.6.

A.3.1.7 Rebaudioside A standard: Rebaudioside A content (mass fraction, on a dry basis) ≥ 99.0%.

A.3.1.8 Rebaudioside D standard: Rebaudioside D content (mass fraction, on a dry basis) ≥ 95.0%.

times of the 13 glycosides.

A.3.4.2 Preparation of standard solutions

Weigh 5.0 mg, 10.0 mg, 20.0 mg, 25.0 mg of rebaudioside A standard and 2.5 mg, 5.0 mg, 10.0 mg, 12.5 mg of rebaudioside D standard, accurate to 0.1 mg, respectively. Place them in a 25 mL volumetric flask. Use acetonitrile aqueous solution to dissolve it. Dilute it to the mark, to obtain the rebaudioside A standard solutions, which have concentrations of 200 mg/L, 400 mg/L, 800 mg/L, 1000 mg/L, AND rebaudioside D standard solutions, which have concentrations of 100 mg/L, 200 mg/L, 400 mg/L, 500 mg/L.

A.3.4.3 Preparation of specimen solution

Weigh 25.0 mg ± 5.0 mg of specimen (dry basis), accurate to 0.1 mg. Put it in a 50 mL volumetric flask. Use acetonitrile aqueous solution to dissolve it. Dilute it to the mark, to obtain a specimen solution.

A.3.5 Determination

A.3.5.1 Drawing of standard curve

Under the reference chromatographic conditions of A.3.3, carry out chromatographic analysis, for 200 mg/L, 400 mg/L, 800 mg/L, 1000 mg/L rebaudioside A standard solution and 100 mg/L, 200 mg/L, 400 mg/L, 500 mg/L rebaudioside D standard solution, respectively. Record the peak area values, corresponding to the standard solutions of each concentration. Take the peak area of rebaudioside A or rebaudioside D, in the chromatogram of the standard solution, as the Y-axis; take the corresponding solution concentration (mg/L) as the X-axis, to draw a standard curve, to obtain the rebaudioside A standard curve and rebaudioside D standard curve, respectively, (forced through the origin). From the standard curve, obtain the rebaudioside A linear function slope (K_a) and the rebaudioside D linear function slope (K_d), respectively.

A.3.5.2 Determination of contents

Under the reference chromatographic conditions of A.3.3, perform chromatographic analysis, on the mixed standard solution and specimen solution, respectively. Compare the chromatogram of the specimen solution with the chromatogram of the mixed standard solution (see Appendix B), to determine the peaks, which are corresponding to each component in the chromatogram of the specimen solution. Record the peak area of rebaudioside A, rebaudioside D, stevia, rebaudioside B, rebaudioside C, rebaudioside E, rebaudioside F, rebaudioside M, rebaudioside N, rebaudioside O, dulcoside A, steviolbioside, in the chromatogram of the specimen solution.

A.3.6 Result calculation

The mass fraction of the 8 glycoside contents (on a dry basis) is calculated, according

A.4.1 Instruments and equipment

PH meter.

A.4.2 Reagents and materials

Weigh 1 g of specimen. Dissolve it in 100 mL of carbon dioxide-free water. Use an acid meter, to measure the pH of the specimen solution.

A.5 Determination of methanol and ethanol

A.5.1 Reagents and materials

A.5.1.1 Methanol: Chromatographically pure.

A.5.1.2 Ethanol: Chromatographically pure.

A.5.1.3 Water: Grade-1 water specified in GB/T 6682.

A.5.2 Instruments and equipment

Gas chromatograph: Equipped with hydrogen flame ionization detector (FID) and headspace sampler.

A.5.3 Reference chromatographic conditions

A.5.3.1 Chromatographic column: Bonded polyethylene glycol fused silica capillary column (column length is 30 m; column inner diameter is 0.25 mm; film thickness is 0.25 μm), or other equivalent chromatographic columns.

A.5.3.2 Carrier gas: Nitrogen (Purity ≥ 99.99%)

A.5.3.3 Carrier gas flow: 5.0 mL/min.

A.5.3.4 Column temperature: Hold at 40 °C for 5 min; heat up to 120 °C, at 10 °C/min; hold for 2 min; finally heat up to 200 °C, at 16 °C/min; hold for 5 min.

A.5.3.5 Sample inlet temperature: 200 °C.

A.5.3.6 Detector temperature: 250 °C.

A.5.3.7 Injection volume: 1 mL.

A.5.3.8 Split ratio: 1:50.

A.5.4 Reference headspace conditions

A.5.4.1 Headspace bottle temperature: 80 °C.

A.5.4.2 Headspace bottle's equilibration time: 30 min.

A.5.5 Analytical procedures

A.5.5.1 Preparation of blank solution

Pipette 2 mL of water. Put it in a headspace bottle. Quickly press tightly the bottle cap. Prepare for use.

A.5.5.2 Preparation of standard solutions

A.5.5.2.1 Preparation of methanol standard solution

Weigh 0.1 g of methanol, accurate to 0.001 g. Use water to dilute it. Transfer it into a 1000 mL volumetric flask. Add water to the mark. Shake well, to obtain 100 mg/L methanol standard stock solution. Prepare this solution, into a series of methanol standard solutions, which have a concentration of 2.5 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 50 mg/L. Pipette 2 mL of each of the above-mentioned series of concentration solutions, place them in headspace bottle. Quickly press tightly the caps, to prepare for later use.

A.5.5.2.2 Preparation of ethanol standard solution

Weigh 0.1 g of ethanol, accurate to 0.001 g. Use water to dilute it. Transfer it into a 100 mL volumetric flask. Add water to the mark. Shake well, to obtain 1000 mg/L ethanol standard stock solution. Prepare this solution, into a series of ethanol standard solutions, which have a concentration of 50 mg/L, 100 mg/L, 200 mg/L, 500 mg/L, 750 mg/L. Pipette 2 mL of each of the above-mentioned series of concentration solutions, place them in headspace bottle. Quickly press tightly the caps, to prepare for later use.

A.5.5.3 Preparation of specimen solution

Weigh 1.0 g of specimen, accurate to 0.001 g. Use water to dissolve it. Transfer it into a 10 mL volumetric flask. Ultrasonicate it for about 3 min, at room temperature. Add water to dilute to the mark. Shake well. Pipette 2 mL of the solution, into a headspace bottle. Quickly press tightly the caps, to prepare for later use.

A.5.6 Determination

Under the reference operating conditions A.5.3 and A.5.4, measure the blank solution, standard series solution, specimen solution, respectively. Record the peak area value of methanol or ethanol. Take the peak area of methanol or ethanol, in the chromatogram of the standard series solution, as the Y-axis; take the corresponding solvent concentration (mg/L) as the X-axis, to draw a standard curve, to obtain a methanol standard curve or an ethanol standard curve. According to the peak area value of methanol or ethanol, in the chromatogram of the specimen solution, obtain the concentration (mg/L) of methanol or ethanol in the specimen solution, from the standard curve.

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