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

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**Determination of tannin content in fruit, vegetable and derived
product. Spectrophotometry method**

水果、蔬菜及其制品中单宁含量的测定 分光光度法

(English Translation)

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Foreword

This Standard was proposed by AND shall be under the jurisdiction of the Ministry of Agriculture of the People's Republic of China.

Drafting organizations of this standard. Quality Supervision, Inspection and Testing Center for Fruits and Seedlings of the Ministry of Agriculture (Xingcheng); the Institute of Fruit Tree Research of the Chinese Academy of Agricultural Sciences.

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Determination of tannin content in fruit, vegetable and derived product - Spectrophotometry method

1 Scope

This standard specifies a method for the determination of tannin content in fruits, vegetables, and derived products, using ultraviolet-visible spectrophotometry.

This standard is applicable to the determination of tannin content in fruits, vegetables, wines.

The detection limit of the method specified in this standard is 0.01 mg/kg; the linear range is 0 mg/L ~ 5.0 mg/L.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For the dated references, the subsequent amendments (excluding corrections) or revisions do not apply to this Standard; however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 8855 Fresh fruits and vegetables - Sampling

3 Principle

Tannin compounds, primarily gallic acid, reduce tungstomolybdic acid to a blue-colored compound in an alkaline solution. This compound exhibits maximum absorbance at 765 nm; its absorbance value is directly proportional to the tannin content. Gallic acid is used as the standard substance; quantification is performed using the standard curve method.

4 Reagents

Unless otherwise specified, distilled water shall be used; all reagents used shall be of analytical purity.

4.1 Sodium tungstate-sodium molybdate mixed solution. Weigh 50.0 g of sodium

tungstate and 12.5 g of sodium molybdate; dissolve them in 350 mL of water in a 1000 mL reflux flask. Add 25 mL of phosphoric acid and 50 mL of hydrochloric acid; mix thoroughly; make it under reflux over a low flame for 2 hours. Subsequently, add 75 g of lithium sulfate, 25 mL of distilled water, a few drops of bromine water; then continue boiling for 15 minutes (until the bromine water has completely evaporated). After cooling, transfer the solution into a 500 mL volumetric flask; make its volume reach to the mark. Filter the solution; store it in an amber bottle; dilute it one fold prior to use. The stock solution can be stored at room temperature for six months.

4.2 75 g/L sodium carbonate solution. Weigh 37.5 g of anhydrous sodium carbonate;

dissolve it in 250 mL of warm water. Mix thoroughly; allow to cool; dilute to 500 mL; filter into a storage bottle for future use.

4.3 Gallic acid standard stock solution. Accurately weigh 0.1100 g of gallic acid monohydrate; dissolve it; dilute it to 100 mL. The mass concentration of gallic acid in this solution is 1000 mg/L. It may be stored in a refrigerator at 2 °C ~ 3 °C for 5 days.

100 mL volumetric flasks; make its volume reach to the mark. The resulting mass

concentrations of gallic acid are 0.0 mg/L, 10.0 mg/L, 20.0 mg/L, 30.0 mg/L, 40.0 mg/L,

6.1 Specimen preparation

Take the edible portion of the fruit and vegetable samples; use clean gauze to wipe away any surface residues. Employ the diagonal division method, to select diagonal sections; chop the sections into small pieces; mix thoroughly; take sample using the quartering method. Homogenize the sample in a tissue homogenizer and set aside for use.

6.2 Tannin extraction

Weigh 2.0 g ~ 5.0 g of the fruit homogenate; wash it into a 100 mL volumetric flask using 80 mL of water. Place the flask in a boiling water bath for 30 minutes for extraction. Remove the flask; allow it to cool; dilute to the mark. Pipette 2.0 mL of the sample extract; centrifuge at 8000 r/min for 4 minutes; set the supernatant aside for use. For wine samples, directly pipette 2.0 mL ~ 5.0 mL; dilute to 100 mL; set aside for use.

6.3 Construction of standard curve

Pipette 1.0 mL each of the gallic acid standard working solutions (4.4) at concentrations of 0.0 mg/L, 10.0 mg/L, 20.0 mg/L, 30.0 mg/L, 40.0 mg/L, 50.0 mg/L; respectively add

3.0 mL of the sodium carbonate solution (4.2). Mix thoroughly. The resulting gallic acid

standard solutions will have concentrations of 0.0 mg/L, 1.0 mg/L, 2.0 mg/L, 3.0 mg/L,

4.0 mg/L, 5.0 mg/L, respectively. Allow the color to develop; let it stand for 2 hours.

Using the 0.0 mg/L on the standard curve as the blank, measure the absorbance of each standard solution at a wavelength of 765 nm. Plot the standard curve, using gallic acid concentration as the abscissa and absorbance values as the ordinate.

6.4 Determination of samples

Pipette 1.0 mL of the specimen extract (6.2); add 5.0 mL of water, 1.0 mL of the sodium tungstate-sodium molybdate mixed solution (4.1), 3.0 mL of the sodium carbonate solution (4.2), respectively, to induce color development. After allowing it to stand for

2 hours, measure the absorbance of the sample solution at a wavelength of 765 nm,

using the 0.0 mg/L on the standard curve as the blank. Determine the tannin concentration in the specimen solution, which is expressed as gallic acid, based on the standard curve. If the absorbance value exceeds the absorbance corresponding to 5.0 mg/L of gallic acid, dilute the sample extract and repeat the determination.

7 Calculation of results

The tannin content in the specimen (expressed as gallic acid) shall be calculated using the formula (1).

Where.

omega - Tannin content in the sample, in milligrams per kilogram (mg/kg) or milligrams per liter (mg/L);

rho - Concentration of gallic acid in the specimen determination solution, in milligrams per liter (mg/L);

10 - Final volume of the specimen determination solution, in milliliters (mL);

A - Dilution factor of the sample;

m - Mass or volume of the specimen, in grams (g) or milliliters (mL).