



National Standard of the People's Republic of China

GB 5413.18-2010

National food safety standard

Determination of vitamin C in foods for infants
and young children, milk and milk products

Preface

This Standard will replace GB/T 5413.18-1997 "Milk powder and formula foods for infants and young children—Determination of vitamin C content".

Compared with GB/T 5413.18-1997, the main amendments of this Standard are as follows:

- The activity unit of enzyme has been defined;
- The concentration of o-phenylenediamine solution was changed;
- The treatment of specimens containing starch was changed;
- The reaction time after boric acid-sodium acetate solution was added.
- The reaction time after o-phenylenediamine solution was added.

This Standard is under the jurisdiction of Ministry of Health of the People's Republic of China.

The releases of all editions substituted by this Standard:

- GB 5413-1985, GB/T 5413.18-1997

National food safety standard

Determination of vitamin C in foods for infants and young children, milk and milk products

1 Scope

This Standard specifies the method for the determination of vitamin C in foods for infants and young children, milk and milk products.

This standard is applicable to the determination of vitamin C in foods for infants and young children, milk and milk products. The determination results indicate the total content of reduction-type and oxidation-type vitamin C.

2 Normative reference

The following normative documents contain provision which, through reference in this text, constitute provisions of This Standard. For dated reference, subsequent amendments to, or revisions of, (excluding mistakes) any of these publications do not apply. However, parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies.

3 Principle

Vitamin C (Ascorbic acid) is oxidized to be dehydroascorbic acid in the presence of activated carbon. The dehydroascorbic acid then reacts with o-phenylenediamine to generate fluorescent substances. The fluorescent spectrophotometer then is applied to determine the fluorescence intensity of the fluorescent substances generated. The fluorescence intensity is proportional to the concentration of ascorbic acid. And the external standard method is used for ration.

4 Reagents and Materials

Unless otherwise specified, the reagents used in the method are the ones of analytically pure, and the water used is the Level 3 Water regulated in GB/T 6682.

4.1 Amylase

Enzyme activity is of 1.5U/mg, the dosage shall be adjusted in accordance with the size of the activity unit.

4.2 Metaphosphoric acid-acetic acid solution A

Dissolve 15g metaphosphoric acid in 200mL water and 40mL acetic acid (36%), then dilute to 500mL with distilled water.

4.3 Metaphosphoric acid-acetic acid solution B

Dissolve 15g metaphosphoric acid in 100mL water and 40mL acetic acid (36%), then dilute to 250mL with distilled water.

4.4 Acid Activated Carbon

Weigh 200g activated carbon (chemically pure, 80 mesh sieve-200 mesh sieve) and then put into 1L hydrochloric acid with the volume fraction of 10%, after that, heat the solution until it boils, then apply the vacuum filtration, after the caking is formed, use a large beaker to hold the caking. Use the water for cleaning the caking until the filtrate is free from any irons. Then use the acid activated carbon after being dried in the oven (5.3) with the temperature of 110 °C-120 °C for 10h.

The method for iron test: Prussian blue reaction. Mix 20g/L potassium ferrocyanide and hydrochloric acid (1%, v/v) and with the equal volume, and then put the above filtrate into the mixture, if the filtrate contains iron, the blue precipitate will be generated.

4.5 Sodium acetate solution

Dissolve 500g sodium acetate trihydrate with the water and then dilute the solution to 1L.

4.6 Boric acid-sodium acetate

Dissolve 3.0g boric acid with the sodium acetate solution (4.5) and dilute to 100mL. The boric acid-sodium acetate shall be prepared before it is required.

4.7 O-phenylenediamine solution (400 mg/L)

Dissolve 40mg o-phenylenediamine and dilute to 100mL with distilled water. The O-phenylenediamine solution shall be prepared when it is required.

4.8 Vitamin C standard solution (100 μ g/mL)

Dissolve 0.050g ascorbic acid and dilute to 50mL with metaphosphoric acid - acetic acid solution A (4.2). Pipette 10.0mL of the above solution and dilute with metaphosphoric acid-acetic acid solution A to 100mL. The Vitamin C standard solution shall be prepared when it is required.

5 Instruments and Equipment

5.1 Fluorescence spectrophotometer

5.2 Scale: Inductance of 0,1 mg.

5.3 Oven

5.4 Incubator: 45 °C \pm 1 °C

6 Analysis Procedures

6.1 Specimen Treatment

6.1.1 Starch-containing Specimen

Weigh 5g solid specimens (with the accuracy of 0.0001 g) evenly mixed or about 20g (with the accuracy of 0.0001 mg) liquid specimen, add 0.1g amylase (4.1) to a 150mL flask. After that, add

the 50mL distilled water with the temperature of 45 -50 ° the solid specimens or add the 30mL distilled water with the temperature of 45 °-50 ° to the liquid specimen. After that, mix evenly and add the nitrogen to the flask to completely discharge the air in the flask, then put the flask with its cover sealed into the oven (5.4) with the temperature of 45 °±1 ° for 30min. After it cools down to the room temperature, put the metaphosphoric acid-acetic acid solution B (4.3) into the 100mL flask and set to the constant volume.

6.1.2 No-starch Specimen

Dissolve the 5g solid specimens evenly mixed (with the accuracy of 0.0001 g) with metaphosphoric acid-acetic acid A (4.2) for dissolving and then set the constant volume to 100mL.

Or dissolve the 50g liquid specimens (with the accuracy of 0.0001 g) with metaphosphoric acid-acetic acid B (4.3) and set the constant volume to 100mL.

6.2 Preparation of Solution to be Determined

6.2.1 Put the above test solution (6.1.1, 6.1.2) and the vitamin C standard solution (4.8) into the 250mL flask with about 2g acidic activated carbon (4.4), then intensely vibrate the flask and filter. Discard the first 5 mL filtrate. Pipette 5.0mL specimen and standard solution and then respectively put into the 25mL and 5.0mL flasks with 50mL boric acid-sodium acetate solution (4.6), place the solution statically for 30min and then add the distilled water to the constant volume. It is the blank solution for specimens and standard solution.

6.2.2 During the 30min, respectively pipette another 5.0mL specimen and standard solution put into the 25mL and 5.0mL flasks with 50mL sodium acetate solution (4.5) and about 15 mL water, and dilute the solution to the regulated scale with distilled water and use as the specimen and the standard solution.

6.2.3 Respectively pipette 2.0mL specimen solution (6.2.2) and the blank solution (6.2.1) of the specimen to 10.0mL tubes and add 5.0mL o-phenylenediamine solution (4.7) to each tube, then shake the tubes uniformly and place in the dark place for 60min before it is used for testing.

6.2.4 Preparation of standard working solution

Respectively pipette 0.5 mL, 1.0 mL, 1.5 mL and 2.0mL above standard solution (6.2.2) into 10 mL test tubes, and dilute them to 2.0 mL with distilled water. At the same time, pipette 2.0 mL the standard blank solution (6.2.1) to a 10 mL test tube. And then add 5.0mL o-phenylenediamine solution (4.7) to each tube, shake the tube uniformly and place in the dark place for 60min before it is used for testing.

6.2 Determination

6.3.1 Preparation of Standard Curve

Immediately put the standard working solution (6.2.4) into the quartz cuvette with the fluorescence spectrophotometer, and then the fluorescence is determined when the solution is of the excitation wavelength of 350nm and the emission wavelength of 430 nm. Draw the standard curve by respectively subtracting the standard blank fluorescence value from the standard fluorescence values, which is the longitudinal coordinates and the corresponding vitamin C content from the standard fluorescence values, which is the horizontal ordinate.

6.3.2 Determination of specimen solution to be determined

The fluorescence value of the specimen solution to be tested (6.2.3) can be obtained by the application of the method in (6.3.1), and then corresponding vitamin C concentration can be obtained in the standard curve by subtracting the fluorescence value of the blank solution from the fluorescence value of the specimen solution.

7 Calculation and Expression of Results

X, the content of the vitamin C in the specimen is expressed by the mass fraction, mg/100g and the equation (1) can be used for calculation:

$$X = \frac{c \times V \times f}{m} \times \frac{100}{1000} \dots\dots\dots (1)$$

Where:

X - The vitamin C content of the specimen, unit: mg/100g;

V - Volume of the specimen solution used in the fluorescent reaction, unit: mL;

c - Mass concentration of vitamin C of the specimen solution from the standard curve, unit: $\mu\text{g/mL}$;

m - Specimen mass, unit: g;

f - The specimen dilution factor.

The final result is the arithmetic mean of the two independent determination results. And the final results must be accurate to the first decimal place.

8 Precision

The absolute difference of the two independent determination results obtained under the repetitive condition shall not exceed 10% arithmetic mean.

9 Others

The detection limit of this standard is 0.1 mg/100g.