GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.84-2016

National food safety standard -

Determination of vitamin B_1 in foods

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Foreword

This standard replaces GB/T 5009.84-2003 “Determination of thiamine (vitamin B_1) in foods”, GB 5413.11-2010 “National food safety standard - Determination of vitamin B_1 in foods for infants and young children, milk and milk products”, GB/T 7628- 2008 “Determination of vitamin B_1 in cereals”, GB/T 9695.27-2008 “Meat and meat products - Determination of vitamin B_1 content”.

As compared with GB/T 5009.84-2003, the main changes of this standard are as follows:

- CHANGE the standard name into “National food safety standard - Determination of Vitamin B_1 in foods”;
- ADD the high performance liquid chromatography as the first method, USE the fluorescence spectrophotometry as the second method;
- MODIFY the expression of detection limit, ADD the quantitative limits of method;
- ADD the qualitative identification method of chloride ion in the artificial zeolite pretreatment;
- ADD the color change characteristics of the solution when using the bromocresol green as the indicator;
- DELETE the Figure 1 (reaction bottle) and Figure 2 (base salt exchange tube) structure;
- ADD the weighing amount of artificial zeolite when it is expressed in wet weight.

National food safety standard -

Determination of vitamin B_1 in foods

1 Scope

This standard specifies the method for determining the vitamin B1 in foods by the high performance liquid chromatography and fluorescence spectrophotometry.

This standard applies to the determination of vitamin B_1 content in food.

Method 1: High performance liquid chromatography

2 Principles

Samples are hydrolyzed, neutralized, then rehydrated in dilute hydrochloric acid medium at constant temperature. The hydrolyzate is derivatized by alkaline potassium ferricyanide solution, extracted with n-butanol, separated by C_{18} reversed-phase column, detected by high performance liquid chromatography-fluorescence detector, quantified by external standard method.

3 Reagents and materials

Unless otherwise indicated, the reagents used in this method are of analytical pure, the water is level I water as specified in GB/T 6682.

3.1 Reagents

3.1.1 n-butanol ($CH_3CH_2CH_2CH_2OH$).

3.1.2 Potassium ferricyanide [$K_3Fe(CN)_6$].

3.1.3 Sodium hydroxide (NaOH).

3.1.4 Hydrochloric acid (HCl).

3.1.5 Sodium acetate ($CH_3COONa \cdot 3H_2O$).

3.1.6 Glacial acetic acid (CH_3COOH).

3.1.7 Methanol (CH_3OH): Chromatographic pure

5.1.3 Other solid samples with lower moisture content: for example, the grain having a moisture content at about 15%, TAKE about 100 g of sample, USE crusher to crush the sample to obtain the power of constant uniformity, MAKE determination in time or PRESERVE it in refrigerator.

5.2 Preparation of specimen solution

5.2.1 Test solution extraction

WEIGH 3 g ~ 5 g (accurate to 0.01 g) of solid sample or 10 g ~ 20 g of liquid sample, PLACE it into a 100 mL conical flask (with a soft stopper), ADD 60 mL of 0.1 mol/L hydrochloric acid solution, SHAKE it uniformly, PLUG the soft stopper, PLACE it in the autoclave at 121 °C for 30 min. After finishing hydrolysis and cooling it to below 40 °C, TAKE it out, SHAKE it gently for several times; USE the pH indicator, USE 2.0 mol/L sodium acetate solution to adjust the pH to about 4.0, ADD 2.0 mL (the consumption amount is appropriately adjusted based on different enzyme activity) of mixed enzyme solution, SHAKE it uniformly; PLACE it in an incubator at 37 °C for overnight (about 16 h); TRANSFER all the enzyme solution into a 100 mL volumetric flask, USE water to make its volume reach to the mark, SHAKE it uniformly, CENTRIFUGE or FILTER it, TAKE the supernatant to prepare for use.

5.2.2 Test solution derivatization

Accurately PIPETTE 2.0 mL of supernatant or filtrate into a 10 mL test tube, ADD 1.0 mL of alkaline potassium ferricyanide solution, VORTEX it uniformly, accurately ADD 2.0 mL of n-butanol, VORTEX it again to mix it uniformly for 5 min, LET it be standing for about 10 min or CENTRIFUGE it; after it is fully stratified, ABSORB the n-butanol phase (upper layer), USE a 0.45 μm organic microporous membrane to filter it, TAKE 2 mL of filtrate and PLACE it into a 2 mL brown sample vial to prepare for analysis. If the concentration of vitamin B_1 in the test solution exceeds the maximum concentration in the linear range, it shall take the supernatant to dilute it for several times for re- derivatization, AND make sample injection again.

TAKE another 2.0 mL of standard series working solution, MAKE it subject to derivatization synchronously with the test solution.

Note 1: Derivatives are stable within 4 h at room temperature.

Note 2: The operation process of 5.2.1 and 5.2.2 shall be avoided in bright light environment.

Note 3: As for the dried pepper and other samples, when the extract is determined after direct derivatization, the recovery rate of vitamin B_1 is relatively low. After the extract is purified by the artificial zeolite, the recovery rate of the

c - The concentration of vitamin B_1 calculated from the standard curve of the test solution (extract), in micrograms per milliliter (μg/mL);

V - Test solution (extract) constant volume, in milliliters (mL);

f - Dilution factor of test solution (supernatant) before derivatization;

m - Mass of the specimen, in grams (g).

The calculation results are expressed as the arithmetic mean of two independent determinations obtained under repeatability conditions, the result is retained with three significant figures.

Note: The measured thiamine content determined in the specimen is multiplied by the conversion factor of 1.121, to obtain the content of thiamine hydrochloride.

7 Precision

The absolute difference between two independent determinations obtained under repeatability conditions shall not exceed 10% of the arithmetic mean.

8 Others

When the amount of weighing sample is 10.0 g, in accordance with the constant volume of this standard method, the detection limit of vitamin B_1 in food is 0.03 mg/100 g, the limit of quantitation is 0.10 mg/100 g.

Method 2: Fluorescence spectrophotometry

9 Principles

Thiamin is oxidized to thiopyrimidine in alkaline potassium ferricyanide solution, under the irradiation of ultraviolet light, the thiopyrimidine fluoresces. Under the given conditions where there is no interference from other fluorescent substance, the intensity of this fluorescence is proportional to the amount of thiopyrimidine amount, i.e., proportional to the amount of thiamine in solution. If the specimen contains too much impurity, it shall use an ion exchanger to treat it to separate the thiamine from the impurities, then use the obtained solution for determination.

Purification of the sample extract: Accurately ADD 20 mL of the above extract into the salt-based exchange column (or chromatographic column), so that the total amount of thiamine of the active artificial zeolite is about 2 μg ~ 5μ g, at a flow rate of about 1 drop/s. ADD 10 mL of near boiling hot water to rinse the salt-based exchange column, at the flow rate of about 1 drop/s, DISCARD the eluent, REPEAT this operation for three times. PLACE at 25 mL graduated test tube below the exchange tube to collect the eluate, ADD 20 mL (divided in two times) of acidic potassium chloride solution at a temperature of about 90 °C, 10 mL for each time of addition at the flow rate of 1 drop/s. After the eluent is cooled to room temperature, USE the 250 g/L acidic potassium chloride to make its volume reach to the mark, SHAKE it uniformly, to obtain the specimen purification solution.

Standard solution treatment: REPEAT the above operation, TAKE 20 mL of vitamin B_1 standard solution (0.1 μg/mL) instead of the specimen extract, same as above, USE the salt-based exchange tubes (or chromatography column) for purification, to obtain a standard purification solution.

12.1.4 Oxidation

ADD 5 mL specimen purification solution into two marked 50 mL centrifuge tubes (A and B, respectively). In the dark, ADD 3 mL of 150 g/L sodium hydroxide solution into the centrifuge tube A, ADD 3 mL of alkaline potassium ferricyanide solution (10.2.9) into the centrifuge tube B, VORTEX it for 15 s; then ADD 10 mL of n-butanol into each tube, VORTEX the tube A and B for 90 s at the same time. LET it be standing for stratification, ABSORB the upper organic phase into another sets of centrifuge tube, ADD 2 g ~ 3 g of anhydrous sodium sulfate, VORTEX it for 20 s, to let the solution be fully dehydrated, PREPARE for determination.

REPEAT the procedure of 12.1.4 with a standard purification solution instead of a specimen purification solution.

12.2 Determination

12.2.1 Fluorescence determination conditions

Excitation wavelength: 365 nm; emission wavelength: 435 nm; slit width: 5 nm.

12.2.2 The following fluorescence intensities are determined in turn

a) Specimen blank fluorescence intensity (specimen reaction tube A);

b) Standard blank fluorescence intensity (standard reaction tube A);

c) Specimen fluorescence intensity (Specimen reaction tube B);

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