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**GB**

NATIONAL STANDARD OF THE  
PEOPLE'S REPUBLIC OF CHINA

**GB 5009.235-2016**

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**National Food Safety Standard -  
Determination of Amino Acid Nitrogen in Foods**

食品安全国家标准

食品中氨基酸态氮的测定

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## Table of Contents

Foreword.....	3
1 Scope.....	4
2 Principle .....	4
3 Reagents and materials.....	4
4 Instruments and apparatuses .....	6
5 Analysis steps .....	6
6 Description of the analysis result.....	7
7 Precision .....	7
8 Principle .....	8
9 Reagents and materials.....	8
10 Instruments and apparatuses .....	9
11 Analysis steps .....	9
12 Description of the analysis result.....	10
13 Precision .....	10
14 Others .....	10

# National Food Safety Standard - Determination of Amino Acid Nitrogen in Foods

## 1 Scope

This Standard specifies the method for determination of amino acid nitrogen in soy sauce, grain paste and soybean paste.

Method 1 of this Standard applies to the determination of amino acid nitrogen in soy sauce which is brewed or prepared from grain and its by-products such as bean cake and bran, sauce which is made from grain, AND soybean paste products which are made from soybean and wheat flour. Method 2 applies to the determination of amino acid nitrogen in soy sauce which is brewed or prepared from grain and its by-products such as bean cake and bran.

## Method 1 - PH meter

## 2 Principle

Use the amphoteric effect of amino acid; add formaldehyde to fix the basicity of the amino group and to make the carboxyl group acidic; use sodium hydroxide standard solution to titrate and fix-volume; use the pH meter to determine the end.

## 3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-3 water that is specified by GB/T 6682.

### 3.1 Reagents

**3.1.1** Formaldehyde (36%~38%): it shall not contain polymer (no precipitation and no stratification of the solution).

**3.1.2** Sodium hydroxide (NaOH).

**3.1.3** Phenolphthalein (C<sub>20</sub>H<sub>14</sub>O<sub>4</sub>).

**3.1.4** Ethanol (CH<sub>3</sub>CH<sub>2</sub>OH).

**3.1.5** Potassium hydrogen phthalate (HOOC<sub>6</sub>H<sub>4</sub>COOH): reference material.

## 4 Instruments and apparatuses

4.1 PH meter (with magnetic stirrer).

4.2 10 mL micro-basic burette.

4.3 Analytical balance: the sensitivity is 0.1mg.

## 5 Analysis steps

### 5.1 Soy sauce sample

Weigh 5.0 g of sample into a 50 mL beaker; use water to wash it into a 100 mL volumetric flask for several times; add water to the scale; mix and take 20.0 mL into a 200 mL beaker; add 60 mL of water; turn on the magnetic stirrer; use sodium hydroxide standard solution [ $c(\text{NaOH}) = 0.050 \text{ mol/L}$ ] to titrate until the pH meter indicates a pH of 8.2; record the number of milliliters of the sodium hydroxide standard titration solution which is consumed to calculate the total acid content. Add 10.0 mL of formaldehyde solution and mix. Use sodium hydroxide standard titration solution to further titrate until the pH reaches 9.2; record the number of milliliters of the sodium hydroxide standard titration solution which is consumed. At the same time, take 80 mL of water; firstly, use sodium hydroxide standard solution [ $c(\text{NaOH}) = 0.050 \text{ mol/L}$ ] to adjust to pH 8.2; then add 10.0 mL of formaldehyde solution; use sodium hydroxide standard titration solution to titrate to pH 9.2; do a reagent blank test.

### 5.2 Grain paste and soybean paste samples

Evenly stir the grain paste or soybean paste sample; place it in a mortar, and rapidly ground it to a state that no visible particles are seen in 10 minutes; place it in a grinding bottle for use. Use a weighing bottle of a known weight to weigh 5.0 g of the sample which is evenly stirred; use 50 mL of distilled water at about 80°C to wash it into a 100 mL beaker for several times; cool it, and transfer it to a 100 mL volumetric flask; use a small amount of water to wash the beaker for several times; merge the cleaning mixture into a volumetric flask; add water to the scale; mix and filter. Pipette 10.0 mL of filtrate; place it in a 200 mL beaker; add 60 mL of water; turn on the magnetic stirrer; use sodium hydroxide standard solution [ $c(\text{NaOH}) = 0.050 \text{ mol/L}$ ] to titrate until the pH meter indicates pH 8.2; record the number of milliliters of sodium hydroxide standard titration solution that is consumed, so as to calculate the total acid content. Add 10.0 mL of formaldehyde solution and mix. Use sodium hydroxide standard titration solution to further titrate until the pH reaches 9.2; record the number of milliliters of the sodium hydroxide standard titration solution which is consumed. At the same time, take 80 mL of water; firstly, use sodium hydroxide standard solution [ $c(\text{NaOH}) = 0.050 \text{ mol/L}$ ] to adjust to pH 8.2; then add 10.0 mL of formaldehyde solution; use sodium hydroxide standard titration solution to titrate to pH 9.2; do a reagent blank test.

Every milliliter of the solution is equivalent to 1.0 mg of ammonia-nitrogen (it can be stably stored in the refrigerator below 10°C for more than 1 year).

**9.3.2** Ammonia-nitrogen standard working solution (0.1 g/L): use a pipette to accurately measure 10 mL of ammonia-nitrogen standard stock solution (1.0 mg/mL) in a 100 mL volumetric flask; add water to dilute to the scale; mix. Every milliliter of this solution is equivalent to 100 µg of ammonia-nitrogen (it can be stably stored in the refrigerator below 10°C for 1 month).

## 10 Instruments and apparatuses

**10.1** Spectrophotometry.

**10.2** Electric constant-temperature water bath (100°C ± 0.5°C).

**10.3** 10 mL glass colorimetric tube with a plug.

## 11 Analysis steps

### 11.1 Sample pretreatment

Weigh 1.00 g of sample into a 50 mL volumetric flask; add water to dilute to the scale; mix well.

### 11.2 Preparation of the standard curve

Accurately extract 0 mL, 0.05 mL, 0.1 mL, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL of ammonia-nitrogen standard working solution (equivalent to 0 µg, 5.0 µg, 10.0 µg, 20.0 µg, 40.0 µg, 60.0 µg, 80.0 µg, 100.0 µg of NH<sub>3</sub>-N) into a 10 mL colorimetric tube respectively. Add 4 mL of sodium acetate-acetic acid buffer solution (pH 4.8) and 4 mL of color developer to each colorimetric tube; use water to dilute to the scale; mix well. Place it in a water bath at 100°C to heat for 15 min; then take it out; after it is cooled to room temperature in the water bath, transfer it to a 1 cm cuvette; use a zero-tube as the reference; measure the absorbance at a wavelength of 400 nm; draw the standard curve or calculate the linear regression equation.

### 11.3 Determination of the sample

Accurately extract 2 mL of sample dilution solution into a 10 mL colorimetric tube. Add 4 mL of sodium acetate-acetic acid buffer solution (pH 4.8) and 4 mL of color developer; use water to dilute to the scale; mix well. Place it in a water bath at 100°C to heat for 15 min; then take it out; after it is cooled to room temperature in the water bath, transfer it to a 1 cm cuvette; use a zero tube as the reference; measure the absorbance at a wavelength of 400 nm. Compare the sample absorbance with the standard curve quantitatively or substitute it into the linear regression equation to calculate the sample

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