



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

GB 5009.124-2016

**National food safety standard - Determination of Amino Acid in
Foods**

食品安全国家标准 食品中氨基酸的测定

(English Translation)

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Foreword

This Standard replaces GB/T 5009.124-2003 Determination of Amino Acid in Foods.

Compared with GB/T 5009.124-2003, this Standard has the major changes as follows.

--- Modify the standard name into "National Food Safety Standard - Determination of Amino Acid in Foods";

--- Expand the applicable scope;

--- Add the limit of detection and quantitative limit of the method;

--- Modify the result calculation formula.

National Food Safety Standard

- Determination of Amino Acid in Foods

1 Scope

This Standard specifies using the amino acid analyzer (ninhydrin post-column derivatization ion exchange chromatography) to determine the amino acid in foods.

This Standard is applicable to determine the amino acid hydrolyzed by acid in foods, there are totally 16 kinds of amino acids such as aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine,

phenylalanine, histidine, lysine, and arginine.

2 Principle

The protein in food is hydrolyzed by hydrochloric acid into free amino acid; after being separated by ion exchange column, it occurs color reaction with ninhydrin solution; then determine the amino acid content through the visible light spectrophotometer.

3 Reagents and Materials

Unless otherwise specified, the reagents used in this method are analytically pure; while water is Class-I water stipulated in GB/T 6682.

3.2.1 Hydrochloric acid solution (6mol/L). take 500mL of hydrochloric acid, dilute with

water to 1000mL, mix evenly.

3.3.1 Mixed amino acid standard solution. standard solution approved by the state or

awarded the standard substance certificate by the state.

3.3.2 16 individual amino acid standard substance. solid with purity $\geq$ 98%.

3.4.1 Mixed amino acid standard stock solution (1 μ mol/mL). separately and accurately take individual amino acid standard substances (accurate to 0.00001g) into the same 50mL beaker; use 8.3mL of 6mol/L hydrochloric acid solution to dissolve;

4.8 Test tube concentrator or parallel evaporator (with supporting 15mL~25mL test

tube).

4.9 Amino acid analyzer. ninhydrin post-column derivatization ion exchange chromatography.

5.1 Specimen preparation

Crush the solid or semi-solid specimen by tissue crusher or grinder; use the homogenizer to homogenize the liquid specimen into homogenate, sealed and frozen

to store; when analyzing, thaw it to use.

5.2 Specimen weighing

For sample with good uniformity such as milk powder, etc., accurately weigh a certain quantity of specimen (accurate to 0.0001g); so that the protein content in the specimen is in the range of 10mg~20mg. For samples with unknown protein content, the protein content in the specimen can be measured firstly. Then place the weighed specimen into the hydrolysis tube.

5.3 Specimen hydrolysis

According to the protein content in the specimen, add 10mL~15mL of 6mol/L hydrochloric acid solution into the hydrolysis tube. For the specimen with high water content and low protein content, such as beverages, fruits, vegetables, etc., firstly add the same volume of hydrochloric acid to mix evenly, after that supplement by 6mol/L hydrochloric acid solution to about 10mL. Continue to add 3 drops ~ 4 drops of phenol into the hydrolysis tube.

5.4.1 Instrument conditions

Inject the mixed amino acid standard working solution into the automatic amino acid analyzer; refer to the test protocol and instrument manual of the amino acid analyzer stipulated in JJG1064-2011; appropriately adjust the instrument operation procedures, parameters, and reagent ratio of buffer solution; confirm the instrument operating conditions.

6.1 Calculation of individual amino acids in mixed amino acid standard stock solution

6.2 Calculation of amino acid content in the sample

The amino acid content in the sample assay solution can be calculated by Formula (2).

7 Precision

The absolute difference between two independent measurement results obtained

under the repeatability conditions shall not exceed 12% of the arithmetic mean.

8 Others

When the specimen is solid or semi-solid, the maximum specimen quantity is 2g; the dissolution volume after drying is 1mL; the detection limits and quantitative limits of each amino acid can refer to Table 3.

Appendix A

Chromatogram

The chromatogram of mixed amino acid standard working solution can refer to Table A.1.

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