GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 5009.124-2016

National Food Safety Standard

– Determination of Amino Acid in Foods

Issued on: December 23, 2016 **Implemented on: June 23, 2017**

Issued by: National Health and Family Planning Commission of PRC;
China Food and Drug Administration.

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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.

3.1 Reagents

3.1.1 Hydrochloric acid (HCl): concentration≥36%, guarantee reagent.

3.1.2 Phenol (C_6H_5OH).

3.1.3 Nitrogen: with purity of 99.9%.

3.1.4 Sodium citrate ($Na_3C_6H_5O_7•2H_2O$): guarantee reagent.

3.1.5 Sodium hydroxide (NaOH): guarantee reagent.

3.2 Reagents preparation

3.2.1 Hydrochloric acid solution (6mol/L): take 500mL of hydrochloric acid, dilute with water to 1000mL, mix evenly.

3.2.2 Refrigerant: mix the commercial salt and ice by the mass ratio of 1:3.

Place the hydrolysis tube into the refrigerant, frozen for 3min~5min; connect to the suction tube of vacuum pump; pump vacuum (close to 0Pa); then inject nitrogen; repeatedly pump vacuum, inject nitrogen, after 3 times, seal the cap or tighten the screw cap under nitrogen injecting state.

Place the sealed hydrolysis tube into 110°C±1°C electric blower thermostat or hydrolysis furnace; hydrolyze for 22h, then take out; cooling off to the room temperature.

Open the hydrolysis tube, filter the hydrolysate into 50mL volumetric flask; use small amount of water to wash the hydrolysis tube for several times; transfer the washing liquid into the same 50mL volumetric flask; finally use water to make constant volume to the scale; shake and mix evenly.

Accurately pipette 1.0mL of filtrate into 15mL or 25mL test tube; use the test tube concentrator or parallel evaporator to reduce pressure to dry at the 40°C~50°C heating temperature environment; after drying, the residuals shall use 1mL~2mL of water to dissolve; then reduce pressure to dry; finally evaporate to dryness.

Add 1.0mL~2.0mL of sodium citrate buffer solution with pH 2.2 into the test tube after drying to dissolve; after shaking and mixing evenly; absorb the solution to get through the 0.22μm filter film; transfer into the instrument sample-injecting bottle; it is the sample assay solution for the measurement of the instrument.

5.4 Determination

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.

5.4.2 Chromatographic reference conditions

a) chromatographic column: sulfonic acid cation resin;

b) detection wavelength: 570nm and 440nm.

5.4.3 Determination of specimen

Inject the same volume of amino acid standard working solution and sample assay solution into the amino acid analyzer; calculate the concentration of amino acid in the sample assay solution through the peak area by external standard method.

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