GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

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National Food Safety Standard - Determination of Volatile Basic Nitrogen in Food

食品安全国家标准

食品中挥发性盐基氮的测定

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National Food Safety Standard - Determination of Volatile Basic Nitrogen in Food

1 Scope

This Standard specifies determination methods for volatile basic nitrogen in food.

This Standard is applicable to determination for volatile basic nitrogen in food that meats are main raw materials, fresh (frozen) animal meats, meat products and processed meat products, animal aquatic products and seafood as well as their conditioning products, pickled egg products such as preserved egg (century egg) and salted egg.

Method One -- Semi-micro nitrogen method

2 Principle

Volatile basic nitrogen refers to that animal food, due to action of enzymes and bacteria, during corruption process, makes protein decompose so as to generate basic nitrogenous substances such as ammonia and amines. Volatile basic nitrogen is volatile. It is distilled in alkaline solution. Use boric acid solution to absorb. Use standard acid solution to titrate and calculate content of volatile basic nitrogen.

3 Reagents and materials

Unless otherwise stated, reagents used in this method are analytically-pure and water is grade-three water specified in GB/T 6682.

3.1 Reagents

3.1.1 Magnesium oxide (MgO).

3.1.2 Boric acid (H_3BO_3).

3.1.3 Trichloroacetic acid ($C_2HCl_3O_2$).

3.1.4 Hydrochloric acid (HCl) or sulfuric acid (H_2SO_4).

4.3 Stopper conical flask: 300 mL.

4.4 Semi-micro nitrogen determination device: as shown in Figure A.1.

4.5 Pipettes: 10.0 mL, 25.0 mL, 50.0 mL.

4.6 Microburette: 10 mL, minimum division is 0.01 mL.

5 Analysis steps

5.1 Semi-micro nitrogen determination device

According to Figure A.1, install semi-micro nitrogen determination device. Before using, clean and check sealing of device.

5.2 Sample processing

For fresh (frozen) meat: remove skin, fat, bones, tendons; take lean portion. For fresh (frozen) seafood and aquatic products: remove shell, skin, head, internal organs, bone spurs; take edible portion; grind and mix well. For finished products, directly grind and mix well. Minced meat, meat powder, dried meat floss, fish meal, dried fish floss, liquid sample can be used directly. For pickled eggs such as preserved egg (century egg) and salted egg, remove eggshell, remove egg membrane; according to a ratio of egg: water = 2:1, add water; use mixer to grind and mix well into homogenate. For fresh (frozen) sample, weigh 20 g of sample. For dried products such as meat powder, dried meat floss, fish meal, dried fish floss, weigh 10 g of sample, to the nearest of 0.001 g. For liquid sample, pipette 10.0 mL or 25.0 mL; place in stopper conical flask; accurately add 100.0 mL of water; shake from time to time; sample is evenly dispersed in sample solution; after 30 min of immersion, filter. For preserved egg sample, salted egg sample, weigh 15 g of egg homogenate (when calculating content, multiply egg homogenate mass by 2/3 and it shall be sample mass), to the nearest of 0.001 g; place in stopper conical flask; accurately add 100.0 mL of trichloroacetic acid solution; shake vigorously for 1 min; place for 15 min; after protein is precipitated, filter. Filtrate shall be used in time. When filtrate cannot be used in time, store in a refrigerator at 0°C~4°C for use. For special sample that is rich in protein gelatin, that is sticky, that is not easy to filter, it may use trichloroacetic acid solution to replace water for experiment. For sample that there are many foams during distillation process, it may add 1~2 drops of de-foaming silicone oil.

5.3 Determination

Add 10 mL of boric acid solution, 5 drops of mixed indicators into receiving bottle. Insert the lower end of condenser tube into liquid surface. Accurately pipette 10.0 mL of filtrate. Inject into reaction chamber from small glass. Use 10

V_0 - total volume of sample solution, in milliliters (mL); V_0 = 100 in this Method;

100 - conversion factor that is used to convert calculation results to milligrams per hundred grams (mg/100g) or milligrams per hundred liters (mg/100mL).

The experimental results are expressed as arithmetic mean values of two independent determination results obtained under repetitive conditions. The result retains three significant digits.

7 Precision

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

Method Two -- Automatic Kjeldahl method

8 Reagents and materials

Unless otherwise stated, reagents used in this method are analytically-pure and water is grade-three water specified in GB/T 6682.

8.1 Reagents

8.1.1 Magnesium oxide (MgO).

8.1.2 Boric acid (H_3BO_3).

8.1.3 Hydrochloric acid (HCl) or sulfuric acid (H_2SO_4).

8.1.4 Methyl red indicator ($C_{15}H_{15}N_3O_2$).

8.1.5 Bromocresol green indicator ($C_{21}H_{14}Br_4O_5S$).

8.1.6 95% ethanol (C_2H_5OH).

8.2 Reagent preparation

8.2.1 Boric acid solution (20 g/L): same as 3.2.2.

8.2.2 Hydrochloric acid standard titration solution (0.1000 mol/L) or sulfuric acid standard titration solution (0.1000 mol/L): same as 3.2.4.

8.2.3 Methyl red ethanol solution (1 g/L): same as 3.2.6.

8.2.4 Bromocresol green ethanol solution (1 g/L): same as 3.2.7.

egg, remove eggshell, remove egg membrane; according to a ratio of egg: water = 2:1, add water; use mixer to grind and mix well into homogenate. For preserved egg sample, salted egg sample, weigh 15 g of egg homogenate (when calculating content, multiply egg homogenate mass by 2/3 and it shall be sample mass). Weigh 10 g of sample for other samples, to the nearest of 0.001 g. Pipette 10.0 mL of liquid sample in distillation tube. Add 75 mL of water. Shake to make sample evenly dispersed. Immerse 30 min.

10.3 Determination

10.3.1 Operate instrument according to requirements of instrument operating instructions. Through cleaning, trail operation, make instrument go into normal test running status. Perform reagent blank determination first to obtain blank value.

10.3.2 Add 1 g of magnesium oxide into distillation tube that has been loaded with well-processed sample. Immediately connect to distiller. According to instrument set conditions and instrument operating instructions, start determination.

10.3.3 When determination ends, timely clean and dredge liquid-adding line and distillation system.

11 Expression of analysis results

The content of volatile basic nitrogen in sample is calculated according to formula (2):

$$X = \frac{(V_1 - V_2) \times c \times 14}{m} \times 100 \qquad \cdots\cdots (2)$$

Where,

X - content of volatile basic nitrogen in sample, in milligrams per hundred grams (mg/100g) or milligrams per hundred liters (mg/100mL);

V_1 - volume of hydrochloric acid or sulfuric acid standard titration solution consumed by testing solution, in milliliters (mL);

V_2 - volume of hydrochloric acid or sulfuric acid standard titration solution consumed by reagent blank, in milliliters (mL);

c - concentration of hydrochloric acid or sulfuric acid standard titration solution, in Moores per liter (mol/L);

16 Analysis steps

16.1 Sample processing

For fresh (frozen) meat: remove skin, fat, bones, tendons; take lean portion. For fresh (frozen) seafood and aquatic products: remove shell, skin, head, internal organs, bone spurs; take edible portion; grind and mix well. For finished products, directly grind and mix well. Minced meat, meat powder, dried meat floss, fish meal, dried fish floss, liquid sample can be used directly. For pickled eggs such as preserved egg (century egg) and salted egg, remove eggshell, remove egg membrane; according to a ratio of egg: water = 2:1, add water; use mixer to grind and mix well into homogenate. For fresh (frozen) sample, weigh 20 g of sample. For dried products such as meat powder, dried meat floss, fish meal, dried fish floss, weigh 10 g of sample. For preserved egg sample, salted egg sample, weigh 15 g of egg homogenate (when calculating content, multiply egg homogenate mass by 2/3 and it shall be sample mass), to the nearest of 0.001 g. For liquid sample, pipette 10.0 mL or 25.0 mL; place in stopper conical flask; accurately add 100.0 mL of water; shake from time to time; sample is evenly dispersed in sample solution; after 30 min of immersion, filter. Filtrate shall be used in time. When filtrate cannot be used in time, store in a refrigerator at 0°C~4°C for use.

16.2 Determination

Apply water soluble glue on edges of diffuser. Add 1 mL of boric acid solution and 1 drop of mixed indicator into central inner chamber of diffuser. In outer chamber of diffuser, accurately add 1.0 mL of filtrate. Cover with frosted glass cover. At notched opening of frosted glass cover and edge of diffuser, only gap that can be inserted with pipette tip or dropper shall be saved. Through frosted glass cover, observe whether water-soluble glue seal is tight. If there is loose seal, it needs re-applying water soluble glue. Then from gap, quickly add 1 mL of saturated potassium carbonate solution. Immediately flat push frosted glass cover. Cover diffusion tight. Gently turn it on table in a circular motion way to make sample solution and saturated potassium carbonate solution completely mix. Place in 37°C ± 1°C incubator for 2 h. Cool to room temperature. Remove cover. Use hydrochloric acid or sulfuric acid standard titration solution (0.0100 mol/L) to titrate. Use 1 methyl red ethanol solution and 5 bromocresol green ethanol solution mixed indicator solutions. End color is till magenta. Use 2 methyl red ethanol solutions and 1 methylene blue ethanol solution mixed indicator solution. End color is till blue purple. Perform reagent blank at the same time.

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