Method of Test for Nitrite in Foods

1. Scope

This method is applicable to the determination of nitrite in the sausage, ham, aquatic products, and other meat products.

2. Method

After hot water extraction, protein precipitation and color reaction, analyte is determined by spectrophotometer.

2.1. Equipment

2.1.1. Water bath

2.1.2. Spectrophotometer: 540 nm.

2.2. Chemicals

Potassium ferrocyanide, reagent grade;
Zinc acetate, reagent grade;
Glacial acetic acid, reagent grade;
Sodium tetraborate, reagent grade;
Hydrochloric acid, reagent grade;
p-Aminobenzenesulfonamide, GR grade;
N-(1-Naphthyl)ethylenediamine dihydrochloride, GR grade;
Sodium nitrite, reference standard.

2.3. Apparatus

2.3.1. Volumetric flask: 100 mL, 200 mL, and 1000 mL.

2.3.2. Filter paper: 15 cm in diameter.

2.3.3. Erlenmeyer flask: 250 mL.

2.4. Reagents

2.4.1. Precipitant I

Dissolve and dilute 106 g of potassium ferrocyanide with deionized water to 1000 mL.

2.4.2. Precipitant II

Dissolve and dilute 220 g of zinc acetate and 30 mL of glacial acetic acid with deionized water to 1000 mL.

2.4.3. Saturated sodium tetraborate solution

Gently heat to dissolve 50 g of sodium tetraborate in 500 mL of deionized water. Cool to room temperature and dilute with deionized water to 1000 mL.

2.4.4. Coloring agent I

Heat to dissolve 2 g of *p*-aminobenzenesulfonamide in 800 mL of deionized water in a water bath. Cool to room temperature and filter with filter paper. Add 100 mL of hydrochloric acid to the filtrate slowly, stir occasionally and dilute with deionized water to 1000 mL.

2.4.5. Coloring agent II

Dissolve and dilute 0.25 g of *N*-(1-Naphthyl)ethylenediamine dihydrochloride with deionized water to 1000 mL. Store in an amber bottle. Prepare freshly before use.

2.4.6. Coloring agent III

Transfer 445 mL of hydrochloric acid into 400 mL of deionized water slowly and dilute with deionized water to 1000 mL.

2.5. Standard solution preparation

Accurately weigh 15 mg of sodium nitrite reference standard that was previously dried at 100℃ for 30 min into a 100-mL volumetric flask. Dissolve and dilute with deionized water to volume as the standard stock solution which is equivalent to 100 μg/mL nitrate (NO_2^-), and then store at 4°C. When to use, mix appropriate volume of standard stock solution, and dilute with deionized water to 0.05-3.0 μg/mL NO_2^- as the standard solutions.

2.6. Standard curve

Accurately transfer 20 mL of standard solution and deionized water (for blank), respectively, into a 100-mL volumetric flask. Add 40 mL of deionized water, 10 mL of coloring agent I and 6 mL of coloring agent III, mix thoroughly, stand for 5 min. Then add 2 mL of coloring agent II, mix thoroughly, stand for 15 min, and add deionized water to exactly 100 mL. Measure the absorbance at 540 nm. Establish the standard curve based on the absorbance measured versus corresponding concentration of the standard solution.

2.7. Sample solution preparation

Transfer about 10 g of the fine-cut sample accurately weighed into an Erlenmeyer flask. Add 5 mL of saturated sodium tetraborate solution and 100 mL of hot water (>80°C), heat in the boiling water bath for 15 min, shake occasionally. Take out, cool to room temperature and add 2 mL of Precipitant I and II, respectively, mix thoroughly. Transfer solution into a 200-mL volumetric flask and dilute to volume with deionized water. Stand at room

temperature for 30 min. Filter with a filter paper and take the filtrate as the sample solution.

2.8. Quantification

Accurately transfer 20 mL of the sample solution and deionized water into a 100-mL volumetric flask, respectively. Proceed as directed in section 2.6 to obtain the absorbance at 540 nm of the sample solution. When the sample solution is colored, accurately transfer 20 mL of the sample solution and deionized water into a 100-mL volumetric flask, respectively. Add 40 mL of deionized water and 6 mL of coloring agent III, mix thoroughly. Dilute to volume with deionized water and measure the absorbance at 540 nm to calibrate. Calculate the amount of nitrite in the sample using the following formula:

The amount of nitrite in the sample (g/kg) = $\frac{C \times V}{M \times 1000}$

C：the concentration of nitrite in the sample solution calculated by the standard curve (μg/mL)

V：the volume of the final sample solution (mL)

M：the weight of the sample (g)

Remark

1. Limit of quantitation (LOQ) of this method is 0.001 g/kg.
2. Further validation should be performed when interference compounds appear in samples.