DETERMINATION OF SODIUM NITRITE (residual NO_2) BY COLORIMETRIC METHOD

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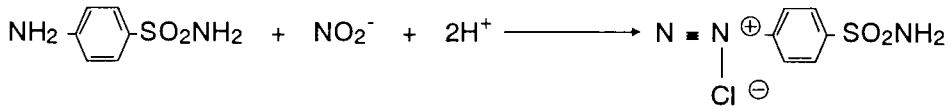
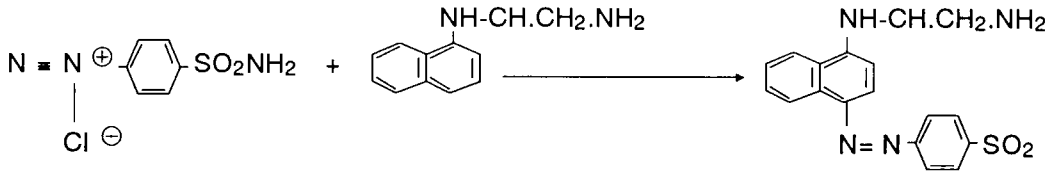
INTRODUCTION

In this method the sulfanilamide combines with the nitrite under acid conditions to become a diazotized salt. The diazotized salt then combines with naphthyl ethylene diamine to form the reddish-violet colour of the azo dye. The intensity of the colour is proportional to the concentration of the nitrite present. The nitrite concentration is determined by the absorbance of the azo dye at a wavelength of 540 nm.

The types of fish products analysed for nitrite are fish sausages, fish hams, salmon roe (or "ikura") and alaska pollack roe.



Sulfanilamide — Diazotized salt



Diazotized salt — Naphthy ethylene diamine — Azo dye

APPARATUS

1. Homogenizer and cup (capacity : 100 ml)
2. Volumetric flasks (25 ml and 200 ml)
3. Water bath (80°C)

REAGENTS

1. 0.5N Sodium hydroxide (NaOH)

2. 12% Zinc sulphate ($ZnSO_4.7H_2O$)

3. 10% Ammonium acetate (CH_3COONH_4) buffer solution :
   Weigh 100 g of ammonium acetate and dissolve in 900 ml of distilled water. Adjust the pH to pH 9.0 using 10% ammonia water (NH_4OH).

4. 1% Ammonium acetate (CH_3COONH_4) buffer solution :
   Dilute the 10% ammonium acetate (pH 9.0) 10 times.

5. 0.5% sulfanilamide (p-aminobenzene sulfonamide, $C_6H_8N_2O_2S$, or sulfanilamidum) : Weigh 0.5 g of sulfanilamide and dissolve in 100 ml of hydrochloric acid with warming (Concentrated HCl : distilled water is 1:1, v/v). Keep in amber glass reagent bottle with glass stopper. This solution can be kept for 4 weeks at 5 - 10°C.

6. 0.12% N-(1-naphthyl)ethylene diamine dichloride ($C_{12}H_{16}Cl_2N_2$).
   Weigh 0.12 g and dissolve in distilled water. Make up to 100 ml. Keep in amber reagent bottle. This solution can be kept for 4 weeks at 5 - 10°C. The solution must be colourless. If there is colour development, the solution should be discarded.

7. HCl (1:1, v/v)
   Dilute concentrated HCl (min. 37%) with an equal volume of distilled water.

8. NO_2 standard solution:

   Preparation of the standard original solution

   Dry sodium nitrite ($NaNO_2$) well in a concentrated sulphuric acid (H_2SO_4) desiccator for 24 hours. Weigh 0.493 g of the dried $NaNO_2$ and dissolve in sterilized, distilled water. Make up to 1000 ml. This is the standard original solution (0.493g $NaNO_2$/1000 ml). Store in amber reagent bottle.

   Preparation of the standard solution.

   Take 10 ml of the standard original solution and make it up to 100 ml with distilled water. Take 2 ml from this and add 10 ml of 1% ammonium acetate. Then make up to 100 ml with distilled water. This is the standard solution. Store in amber reagent bottle. Unstable reagent, must not be stored for more than 2 weeks.

   1 ml of standard sodium nitrite solution contains 0.2 ug NO_2-N.

Preparation of standard curve

Pipette 2.5, 5, 10, 15 and 20 ml of the standard sodium nitrite solution into 25 ml volumetric flasks. Make up to 20 ml with 1% ammonium acetate buffer solution.

These 20 ml solutions contain 0.5, 1, 2, 3 and 4 ug NO_2 respectively.

Spectrophotometric readings of standard curve

Add 1 ml 0.5% sulphanilamide solution into each of the volumetric flasks containing the standard solutions. Shake well. Then add 1 ml 0.12% naphthylethylene diamine solution and shake. Make up to 25 ml with distilled water and shake thoroughly. Let stand for 20 min and read at 540 nm.

PROCEDURE

Sample Preparation (Fig. 1)

1. Weigh 10.0g sample into homogeniser cup.
2. Add about 20 ml to 30 ml of distilled water (ca. 80°C).
3. Homogenize for 30 sec.
4. Pour into 250 ml conical flask.
5. Wash homogenizer cup with distilled water, and make up to 150 ml with hot distilled water (ca. 80°C).
6. Pipette 10 ml of 0.5N NaOH, followed by 10 ml of 12% $ZnSO_4.7H_2O$.
7. Cover the mouth of the flask with aluminium foil and heat for 20 min. at 80°C in a water bath.
8. Cool to room temperature with cool running water.
9. Add 20 ml of 10% NH_4CH_3COOH buffer solution.
10. Make up to 200 ml with distilled water in a volumetric flask.
11. Mix well and stand for 10 min.
12. Filter through Whatman No. 1 filter paper, discarding about 10 ml of first filtrate.
13. Use filtrate as test solution.
14. For a blank test use 10 ml of distilled water instead of sample and repeat steps 1 to 13 to obtain a blank test solution.

Analytical Procedure (Fig. 2)

1. Pipette 20 ml of sample test solution into a 25 ml volumetric flask.
2. Pipette 1 ml of 0.12% naphthylethylene diamine solution into the volumetric flask.
3. Follow up with 1 ml 0.5% sulfanilamide.
4. Make up to 25 ml with distilled water, mix well and stand for 20 min.
5. Measure the absorbance for the colour developed using a spectrophotometer at a wavelength of 540 nm.
6. For a blank test, pipette 20 ml of blank test solution into a 25 ml volumetric flask, and repeat steps 2 to 4.
7. Into a third 25 ml volumetric flask, pipette 20 ml of sample test solution, followed by 1 ml of HCl(1:1, v/v). Then repeat steps 4 and 5.

CALCULATION

NO_2 (ppm) = 3.28 A

A = Aa – (Ab + Ac)

where Aa = Absorbance of sample test solution

Ab = Absorbance of blank test solution

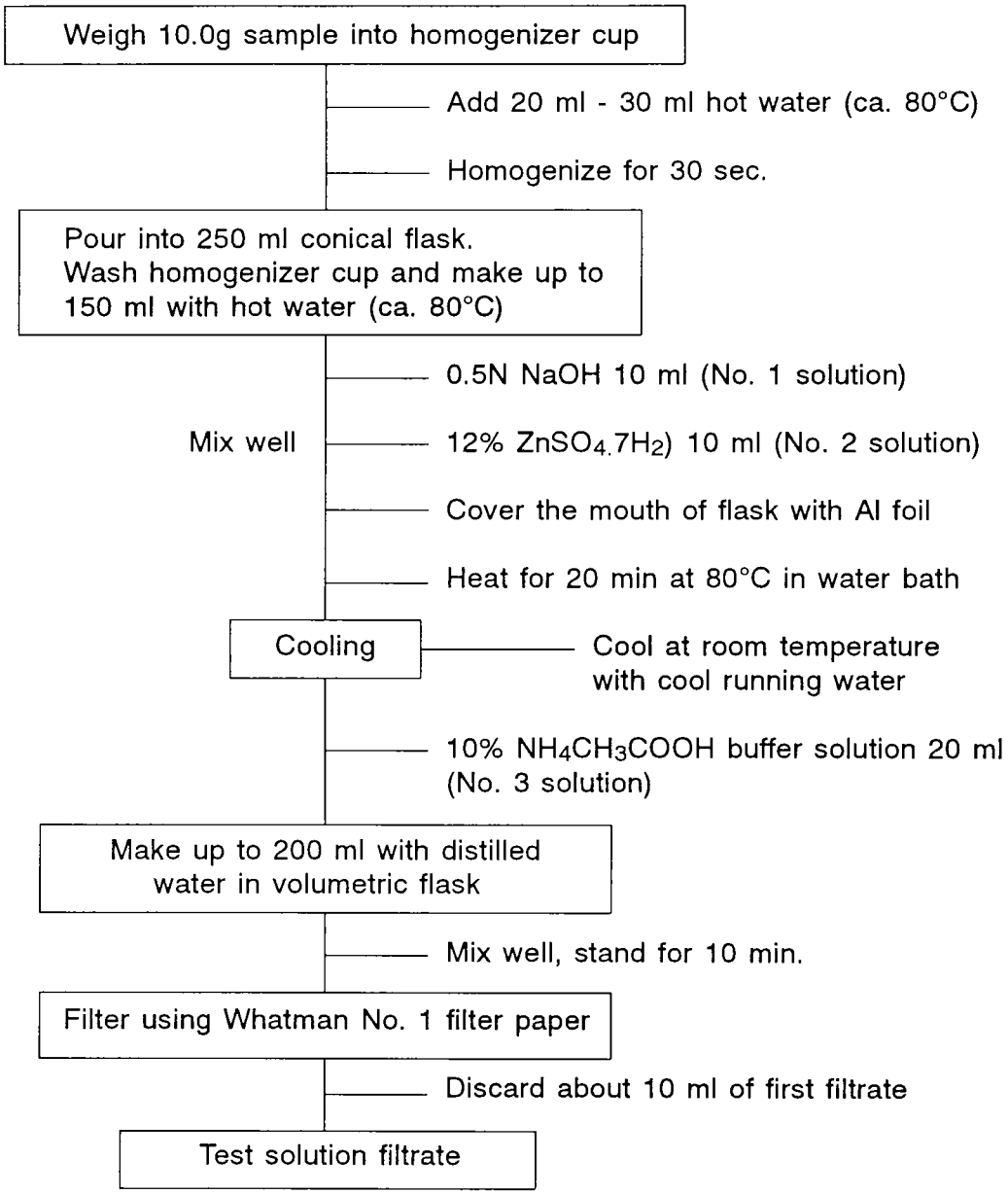
Ac = Absorbance of sample test solution and HCl (1:1)

REMARKS

1. In cases where the fat content is high such as salmon roe and cheese, the filtrate may be turbid. Thus 10 ml of 1N NaOH should be added with 24% $ZnSO_4.7H_2O$ or 20 ml of 0.5N NaOH and 12% $ZnSO_4.7H_2O$. The filtrate will become clear or transparent after this treatment.
2. Detection limit : 0.1 ppm (0.0001 g/kg)

REFERENCE

Shokuhin Eisei Kensa Shishin (I), Guidelines for Food Hygiene Inspection (I), Japan Food Hygiene Association (1973).



N.B. Blank test should be done using 10 ml of distilled water instead of sample. Repeat the above procedure with the blank.

Fig. 1 Sample Preparation for NO_2 analysis

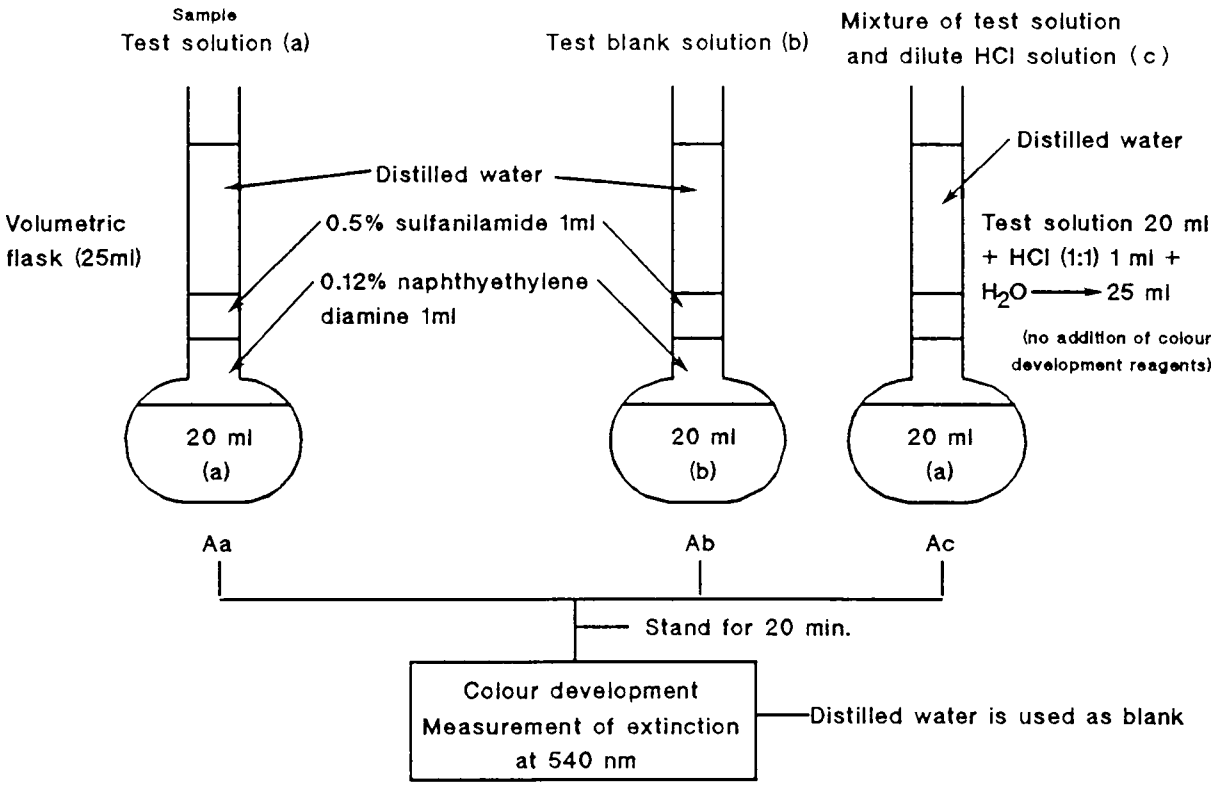


Fig. 2 Analytical Procedure for NO_2 analysis