DETERMINATION OF SULPHUR DIOXIDE (residual SO_2) IN FROZEN SHRIMPS BY COLORIMETRIC AND CONWAY'S MICRODIFFUSION METHOD

MAKOTO YAMAGATA AND LOW LAI KIM

INTRODUCTION

This method uses a combination of colorimetric method and Conway's microdiffusion method. By regulation (under the Food Sanitation Law, Japan), peeled shrimps should have a residual SO_2 of less than 100 ppm (0.1g/kg).

APPARATUS

1. Volumetric flasks (100 ml, 250 ml, 1000 ml)
2. Conway Microdiffusion Units with covers.
3. Pipettes (1 ml, 5 ml, 10 ml, 20 ml)
4. Mortar and pestle
5. Centrifuge (4,000 rpm)
6. Centrifuge tubes
7. Oven (37°C)

REAGENTS

1. Absorbent solution (Mercuric chloride* solution of SO_2)

   Weigh 27.2 g of mercuric chloride ($HgCl_2$) and 11.7 g of sodium chloride (NaCl) and dissolve in an adequate volume of 5% glycerine solution in a volumetric flask. Make up to 1000 ml with 5% glycerine. Then add 0.03 g of sodium azide (NaN_3) to the solution.

2. Diluting solution

   Take one volume of the absorbent solution and mix thoroughly with three volumes of distilled water (1:3, v/v).

   *Caution : Mercuric chloride should be handled with care because it is highly toxic. Proper disposal method for mercuric chloride should be used.

3. ρ-rosaniline formalin solution

   Dissolve 0.2 g of ρ-rosaniline hydrochloride into 100 ml of distilled water and filter if necessary. Measure 20 ml of this solution into a volumetric flask (100 ml) and add 6 ml of HCl solution (hydrochloric acid :12N, 36%). Make up to 100 ml with distilled water. Shake the diluted solution well. Transfer to a 250 ml flask and add 100 ml of 0.2% of formalin (HCHO).

N.B. ρ-rosaniline solution and 0.2% of formalin should be stored separately and mixed on the day of use. It is recommended that 0.2% formalin should be prepared fresh on the day of use.

4. Sulphurous standard solution

Weigh 0.5 g of sodium bisulphite ($NaHSO_3$) and dissolve in distilled water in a 100 ml volumetric flask. Make up to 100 ml with distilled water. This solution contains 5 mg $NaHSO_3$ / ml.

Take 10 ml of the sulphurous standard solution and add 15 ml of 0.1N iodine solution. Then add 2 ml conc. HCl (12N, 36%). Titrate against 0.1N sodium thiosulphate solution. Note the volume of titrant as **a** ml.

For blank use 10 ml of water and add 15 ml of 0.1N iodine solution. Then add 1 ml of conc. HCl (12N, 36%) and titrate against 0.1N sodium thiosulphate solution. Record as **b** ml.

$$A = \frac{93.75}{b - a} \times \frac{1}{f}$$

where :

a = volume of 0.1N sodium thiosulphate (ml) used in titration of sulphurous standard solution

b = volume of 0.1N sodium thiosulphate (ml) used in titration of blank

f = factor of 0.1N sodium thiosulphate solution used

Take A ml of the sodium bisulphite solution and make up to 300 ml with the diluting solution. This is the original sodium bisulphite solution.

5. Standard solution

Pipette 1 ml of the original sodium bisulphite solution into a 100 ml volumetric flask. Make up to 100 ml with diluting solution. Shake thoroughly. This gives the standard sodium bisulphite solution and contains 0.001 mg SO_2 in 1 ml.

PROCEDURE

Sample Preparation

1. Semi-thaw the frozen shrimps.
2. Wash slightly in running water.
3. Peel the shrimps, removing the carapace, shell and tail.
   (**N.B.** Do not wash shelled shrimps).
4. Weigh 10g of minced sample into mortar.
5. Pipette 70 ml of diluting solution followed by 20 ml of saturated (8%) mercuric chloride into mortar.
6. Mince and grind peeled shrimp in the mortar.
7. Centrifuge at 4,000 rpm for 10 min. or filter through Whatman No. 1 filter paper.
8. Collect the supernatant or filtrate as the sample solution.

Analytical Procedure (Fig. 1.)

1. Pipette 1 ml of absorbent solution into inner ring of Conway unit.
2. Pipette 1 ml of sample solution into outer ring of Conway unit.
3. Pipette 0.2 ml of 25% phosphoric acid solution into the outer ring.
4. Place fixing reagent (85% phosphoric acid or white vaseline) on cover of Conway unit.
5. Cover the dish and tighten with clip.
6. Stand at 37°C for 90 min or 30°C for 100 min or 20°C for 2 hours.
7. Pipette sample solution from inner ring into absolute test tube.
8. Wash inner ring with absorbent solution and make up to 5 ml with the absorbent solution.
9. Pipette 1 ml of ρ-rosaniline formalin solution into absolute test tube, mix well and stand at 20° - 25°C for 35 min.
10. Measure the absorbance of the colour developed at a wavelength of 560 nm.

CALCULATION

SO_2 in shrimp muscle(ppm)

$$= \frac{5 \times A \times \text{Total sample solution (100 ml)}}{As \times \text{Sample weight (10 g)}}$$

$$= \frac{5 \times A \times 100}{As \times 10}$$

$$= \frac{A}{As} \times 50$$

or SO_2 in shrimp muscles (g/kg)

$$= \frac{A}{As} \times 0.05$$

Where A = – log T (5 ml sample solution)
As = – log T (5 ml of standard solution)
Blank solution is 5 ml of absorbent solution.

REMARKS

The detection limit : 5 ppm (0.005 g/kg)

REFERENCE

Kanshoku No. 110. Ministry of Health and Welfare, Japan. 24th May 1973.

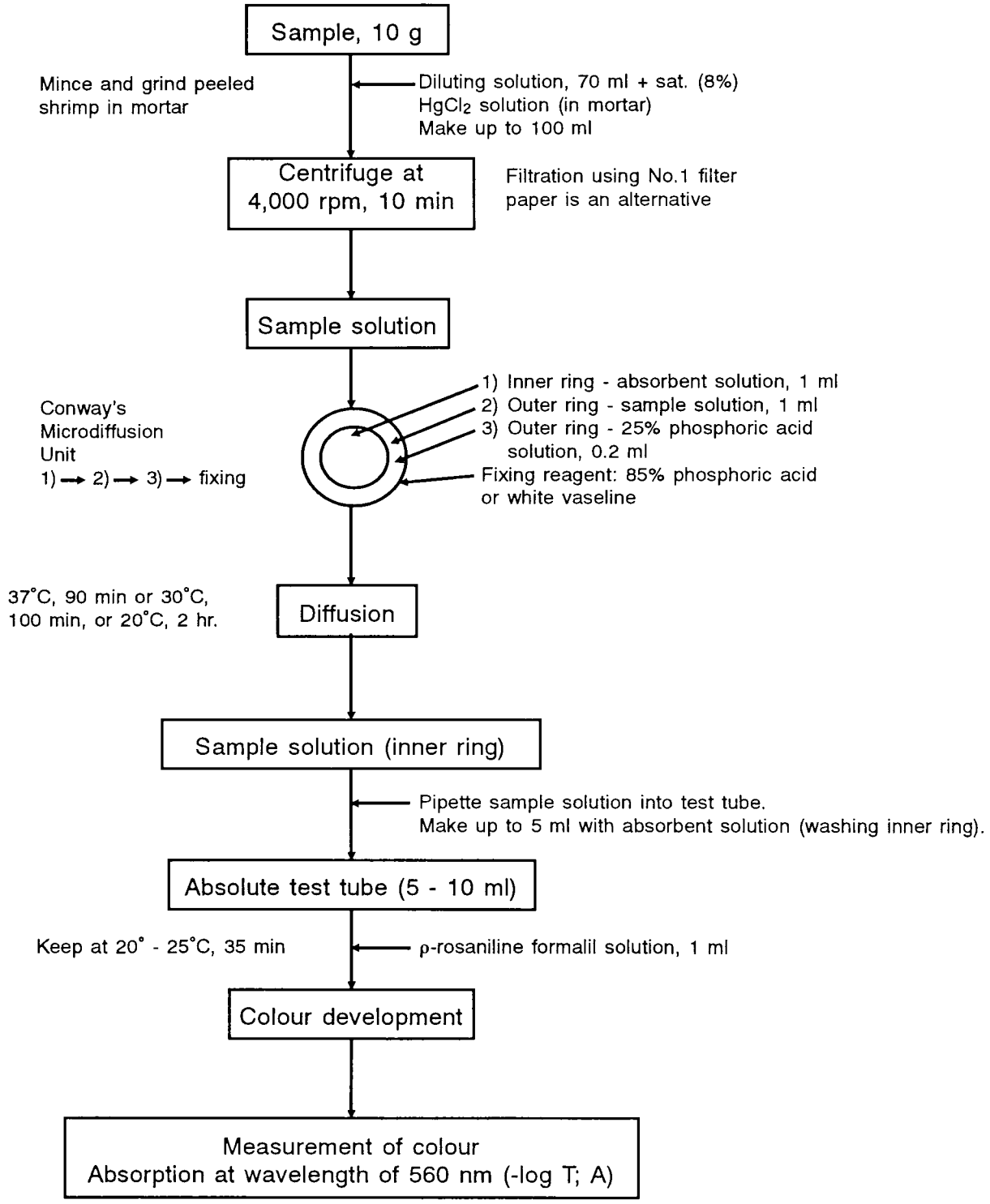

Fig. 1 Analytical procedure for SO_2 analysis