DETERMINATION OF TRIMETHYLAMINE OXIDE (TMAO-N), TRIMETHYLAMINE (TMA-N), TOTAL VOLATILE BASIC NITROGEN (VB-N) BY CONWAY'S MICRODIFFUSION METHOD

(1% Boric acid and 0.02N Hydrochloric acid)

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INTRODUCTION

Trimethylamine oxide (TMAO) is a nitrogenous compound commonly present in marine organisms. It has been suggested that TMAO functions as an osmoregulator in these animals. The degradation of TMAO into simpler compounds such as trimethylamine (TMA), dimethylamine (DMA) and formaldehyde (FA) depends on the enzymes present in the tissue.

Generally, TMAO breaks down to TMA in marine fishes, either by endogenous enzymes, bacteria enzymes or both. However in gadoid fishes, the TMAO is broken down to DMA and FA.

The use of TMA as an index of fish freshness was first proposed by Beatty and Gibbons (1936). This was based on the observation that the production of TMA was dependent on bacterial activity, and the role of autolysis was negligible. The source of TMA was derived both from bacterial activity as well as from endogenous enzymes.

In recent years, there are opinions that TMA itself may not be a very suitable freshness index. This is because the TMA content in a fish may vary with season, and also the distribution of TMA within a piece of fillet may not be uniform. Under the local conditions, TMA was found to be a good indicator of freshness for white pomfret, chinese pomfret and grouper. TMA is not a good indicator of freshness for lizard fish. Instead, DMA and FA are suitable indices.

The total volatile basic substances (VB-N) in fish meat is mainly composed of ammonia, TMA, and DMA. The level of VB-N increases after spoilage begins (both enzymatic and bacterial). It does not distinguish the origin nor component of these volatile compounds, hence its use is more general.

In this laboratory, the microdiffusion method devised by Conway is adopted. In this method, TMA, TMAO and VB-N are determined as their nitrogen. To obtain the actual amount of TMA or TMAO, the nitrogen values must be divided by the amount of nitrogen present per molecule of TMA or TMAO.

Principle of the Conway unit in determination of VB-N

The solution in the inner ring of the Conway unit contains 1% solution of boric acid with bromocresol green and methyl red indicator. The sample extract is in the outer ring. On the addition of K_2CO_3 the sample extract becomes alkaline. The TMA and related compounds present in the sample extract are released under alkaline condition as volatile compounds. The volatile compounds diffuse into the boric acid solution to form boric acid salt of these compounds. These salts are reduced to HCl-salts by a strong acid (HCl) during titration.

APPARATUS

1. Conway's unit:

   Wash with detergent (use neutral detergent if available), then rinse with running water and dry in air. Do not wipe with cloth.

2. Micro-burette (to 4 decimal places)

3. Oven (37°C)

4. Volumetric flasks, 10 ml, 100ml, 1000 ml

5. Pipettes, 1 ml, 10 ml

6. Mortar and pestle

7. Centrifuge, 3,000 rpm

8. Centrifuge tubes

9. Weighing balance

REAGENTS

1. Inner ring solution - 1% boric solution containing indicator

   Weigh 10 g of boric acid into 1 litre volumetric flask then add 200 ml of ethanol. After dissolving the boric acid, add 10 ml of mixed indicator solution, then make up to 1 litre with distilled water.

2. Mixed indicator solution

   Dissolve bromocresol green (BCG) 0.01 g and methyl red (MR) 0.02 g in 10 ml of ethanol.

3. 0.02N Hydrochloric acid, HCl

   Dilute 20 ml of 1N HCl standard solution with distilled water and make up to 1000 ml.

4. Saturated potassium carbonate (K_2CO_3) solution

   Weigh 60 g of K_2CO_3 and add 50 ml of distilled water. Boil gently for 10 min. After cooling down, filter through filter paper.

5. 50% potassium carbonate (K_2CO_3) solution

   Dilute saturated K_2CO_3 solution to twice its volume with distilled water.

6. 4% Trichloroacetic acid (TCA, CCl_3COOH) solution:

   Dissolve 40 g of TCA in 960 ml of distilled water.

7. Sealing agent

   Weigh 3 g of Tragacanth gum. Add 30 ml of distilled water, 15 ml of glycerine and 15 ml of 50% saturated K_2CO_3 solution and mix well.

8. Neutralized 10% formaldehyde solution

   Add 10 g of $MgCO_3$ to 100 ml of formalin (35% formaldehyde solution) and shake in order to neutralize the acidity of formalin. Filter and dilute the filtrate 3 times with distilled water.

9. 1% Titanium trichloride ($TiCl_3$) aqueous solution*

   Pipette 6.7 ml of 15% $TiCl_3$ into 100 ml volumetric flask and make up to 100 ml with distilled water.

10. Saturated potassium nitrate (KNO_3) aqueous solution

    Dissolve about 55 g of KNO_3 in 50 ml of distilled water.

* If the stock $TiCl_3$ has been stored for sometime, a recovery test using various concentrations (%) of $TiCl_3$ should be carried out and the appropriate percentage yielding close to 100% recovery should be used.

PROCEDURE
(Refer to Fig. 1)

Sample Preparation

1. Weigh 2 g of fish meat in a mortar and grind well.
2. Add 8 ml of 4% TCA solution and grind well.
3. Stand for 30 min at ambient temp. with occasional grinding.
4. Filter through filter paper (Whatman No. 1) or centrifuge at 3000 rpm for 10 min.
5. Keep the filtrate (sample solution) in -20°C freezer if necessary.

Analytical Procedure

1. Determination of VB-N

   1.1 Apply sealing agent to Conway's unit.

   1.2 Pipette 1 ml of inner ring solution into inner ring.

   1.3 Pipette 1 ml of sample solution into outer ring.

   1.4 Slant the Conway's unit with cover.

   1.5 Pipette 1 ml of saturated K_2CO_3 solution into outer ring.

   1.6 Immediately close the unit and tighten with clip.

   1.7 Mix the outer ring solutions gently.

   1.8 Stand for 60 min at 37°C in incubator.

   1.9 Titrate inner ring solution against 0.02N HCl using a micro-burette until green colour turns pink.

   1.10 Do blank test using 1 ml of 4% TCA instead of sample solution.

2. Determination of TMA-N

2.1 Apply sealing agent to Conway's unit.

2.2 Pipette 1 ml of inner ring solution into inner ring.

2.3 Pipette 1 ml of sample extract into outer ring.

2.4 Pipette 1 ml of neutralized 10% formaldehyde into outer ring and gently mix the outer ring solutions.

2.5 Slant the Conway's unit with cover.

2.6 Pipette 1 ml of saturated K_2CO_3 solution into outer ring.

2.7 Immediately close the unit and tighten with a clip.

2.8 Mix the outer ring solutions gently.

2.9 Stand for 60 min at 37°C in incubator.

2.10 Titrate inner ring solution against 0.02N HCl using a micro-burette until green colour turns pink.

2.11 Do blank test using 1 ml of 4% TCA instead of sample solution.

3. Determination of TMAO-N

3.1 Take 2 ml of the sample solution into a test tube.

3.2 Add 1 ml of 1% $TiCl_3$ and fully mix. The solution will turn violet.

3.3 Stand in a 80°C water bath for 90 sec. The violet colour should disappear.

3.4 Add saturated KNO_3 dropwise in cases where violet colour persists until it disappears.

3.5 Cool in running water.

3.6 Transfer the solution to a 10 ml volumetric flask.

3.7 Make up to 10 ml with washings and distilled water.

3.8 Proceed as for TMA-N determination

CALCULATION

TMA-N or VB-N (mg/100g)	=	Amt. of HCl used in titration	X	Amt. of ammonium nitrogen equivalent to 1 ml of 0.02N HCl	X	Ratio of the amt. of sample used to 100g muscle

$$= (V_S - V_B) \times (N_{HCl} \times A_N) \times \frac{[(W_S \times \frac{M}{100}) + V_E] \times 100}{W_S}$$

TMAO-N (mg/100g) = (TMA-N after $TiCl_3$ reduction X 5) - (TMA-N before $TiCl_3$ reduction)

where, V_S = Titration volume of 0.02N HCl for sample extract (ml)

V_B = Titration volume of 0.02N HCl for blank (ml)

N_{HCl} = Normality of HCl (=0.02N x f, factor of HCl)

A_N = Atomic weight of nitrogen (14.00)

W_S = Weight of muscle sample (g)

M = Percentage moisture of muscle sample

V_E = Volume of 4% TCA used in extraction

N.B. 1 ml of 0.02N HCl = 0.28 ammonia nitrogen

= $(N_{HCl} \times f \times 14.00)$

Detection limit : 0.2 mg/100g

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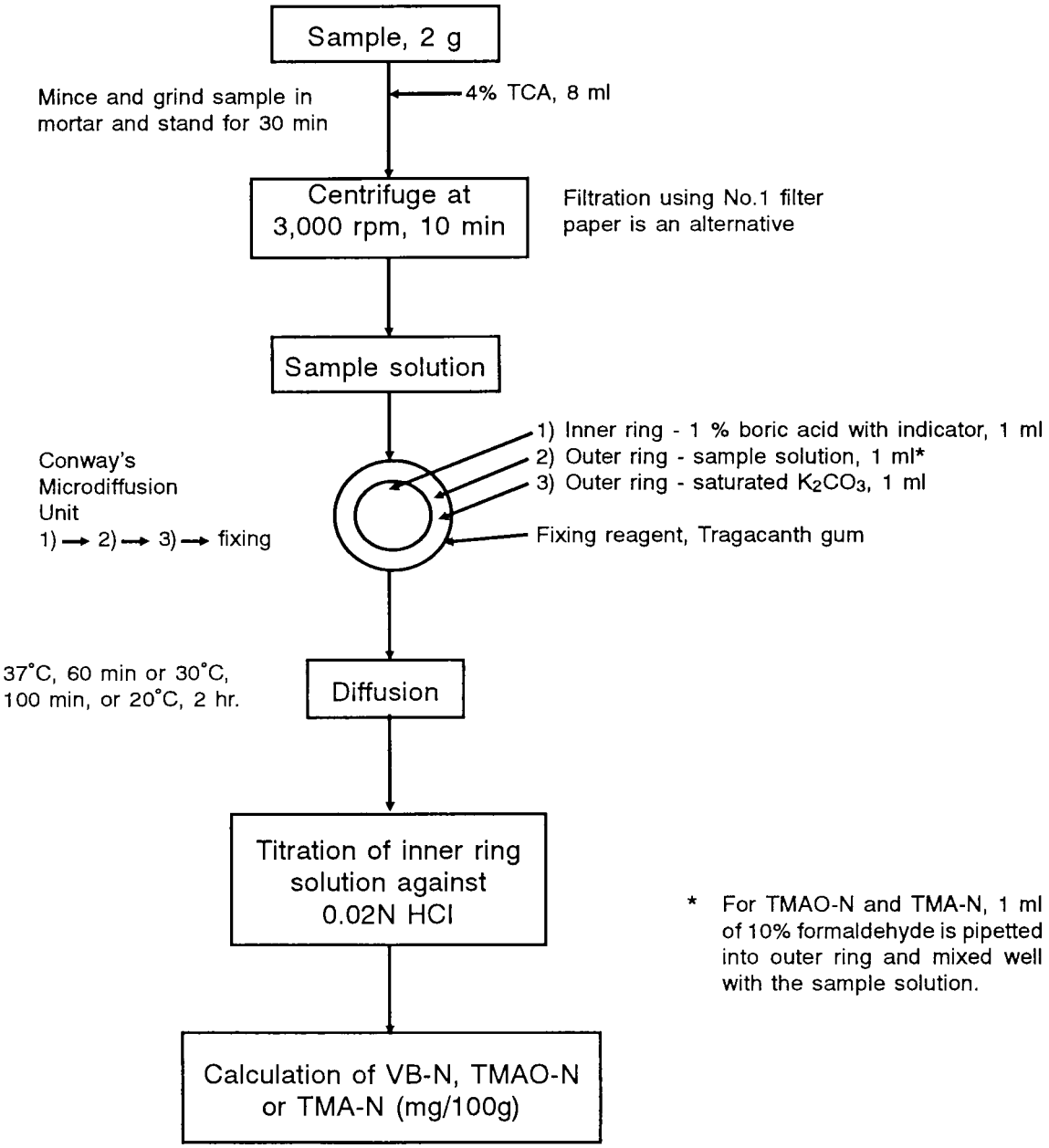

Fig. 1 Analytical procedure for VB-N, TMAO-N, TMA-N analysis