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

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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 bacteria activity, and the role of autolysis was negligible. The source of TMA in ordinary muscle is due to the bacterial degradation of TMAO to TMA, while in dark muscles, TMA was derived both from bacteria 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, grouper and siakap. TMA is not a good indicator of freshness for lizard fish. Instead, DMA and FA are suitable indices.

The total volatile basic substances (TVB) in fish meat is mainly composed of ammonia, TMA, and DMA. The level of TVB 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 TVB 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 TVB

The solution in the inner ring of the Conway unit contains a 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 in 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.

I REAGENTS

a) Inner ring solution — 1% boric acid solution containing indicator:

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

b) Mixed indicator solution:

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

c) 0.02N HCl:

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

d) Saturated K_2CO_3 solution:

Take 60 g of potassium carbonate (K_2CO_3), and add 50 ml of distilled water. Boil gently for 10 min. After cooling down, obtain filtrate through filter paper.

e) 50% K_2CO_3 solution:

Dilute saturated K_2CO_3 solution twice with distilled water.

f) 4% trichloroacetic acid (CCl_3COOH) (TCA) solution:

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

g) Sealing agent:

Take 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.

h) 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 filtrate 3 times with distilled water.

i) 1% $TiCl_3$ aqueous solution:

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

j) Saturated KNO_3 aqueous solution:

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

II APPARATUS

Conway's unit:

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

Micro-burette

III PROCEDURE

A. SAMPLE EXTRACTION

1. Take 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 occassional grinding.
4. Filter through filter paper (Whatman No. 41). (or Centrifuge at 3000 rpm, for 10 min.)
5. Keep the filtrate in −20°C freezer if necessary.

B. DETERMINATION OF TVB-N

1. Apply sealing agent to Conway's unit.
2. Pipette 1 ml of inner ring solution into inner ring.
3. Pipette 1 ml of sample extract into outer ring.
4. Slant the Conway's unit with cover.
5. Pipette 1 ml of saturated K_2CO_3 solution into outer ring.
6. Close the unit.
7. Mix gently.
8. Stand for 60 min. at 37°C in incubator.
9. Titrate inner ring solution with o.o2N HCl using a micro-burette until green colour turns to pink.
10. Do blank test using 1 ml of 4% TCA instead of sample extract.

C. DETERMINATION OF TMA-N

Principle of TMA-N determination is similar to TVB-N determination except addition of formaldehyde to the sample solution. Formaldehyde is added in order to fix any ammonia present in the sample.

1. Apply sealing agent to Conway's unit.
2. Pipette 1 ml of inner ring solution into inner ring.
3. Pipette 1 ml of sample extract into outer ring.
4. Pipette 1 ml of neutralized 10% formaldehyde into outer ring.
5. Slant the Conway's unit with cover.
6. Pipette 1 ml of saturated K_2CO_3 solution into outer ring.
7. Close the unit.
8. Mix gently.

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

10. Titrate inner ring solution with 0.02N-HCl using a micro-burette until green colour turns to pink.

11. Do blank test using 1 ml of 4% TCA instead of sample extract.

CALCULATION OF TMA-N OR TVBN

$$\text{TMA-N or TVBN (mg/100 g)} = \left(\begin{array}{l}\text{Amt. of HCl}\\ \text{used in}\\ \text{titration}\end{array}\right) \times \left(\begin{array}{l}\text{Amt. of ammonium}\\ \text{nitrogen equivalent to}\\ \text{1 ml of 0.02N HCl}\end{array}\right) \times \left(\begin{array}{l}\text{Ratio of the amount}\\ \text{of sample used to}\\ \text{100 g muscle}\end{array}\right)$$

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

where

V_s = Titration volume of 0.02N HCl for sample extract (ml)

V_B = Titration volume for blank (ml)

N_{HCl} = Normality of HCl (=0.02N × 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

NOTE: 1 ml of 0.02N HCl = 0.28 ammonium nitrogen
= (N_{HCl} × f × 14.00)

D. DETERMINATION OF TMAO-N

Reduction of sample extract

1. Take 2 ml of the filtrate (sample extract) or its dilute into a test tube.

2. Add 1 ml of 1% $TiCl_3$ and fully mix, then confirm that pink colour does not disappear.

3. Stand in an 80°C water bath for 90 sec.

4. Add saturated KNO_3 dropwise until the pink colour disappears.

5. Cool in water.

6. Transfer the solution to 10 ml volumetric flask.

7. Make up to 10 ml with distilled water.

8. Proceed as for TMA-N determination.

CALCULATION OF TMAO-N

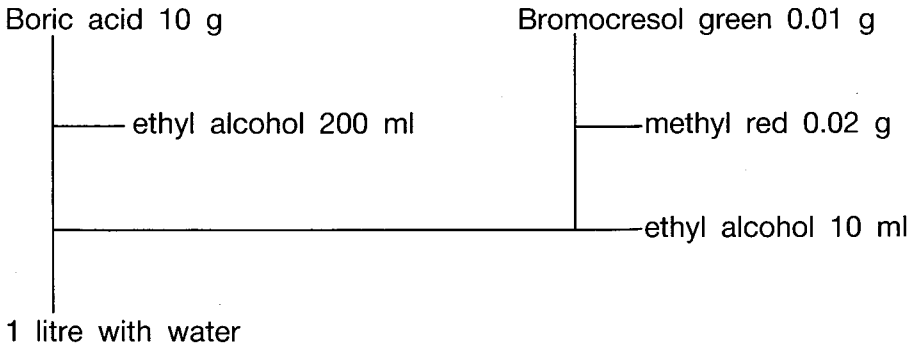
TMAO-N = (TMA-N after $TiCl_3$ reduction)* — (TMA-N before $TiCl_3$ reduction)

* Care must be taken to obtain the correct dilution factor since in TMAO-N determination, the sample was made up to 10 ml before applying into the Conway unit.

ANNEX I. SCHEMATIC FORM FOR PREPARATION OF REAGENTS

REAGENTS

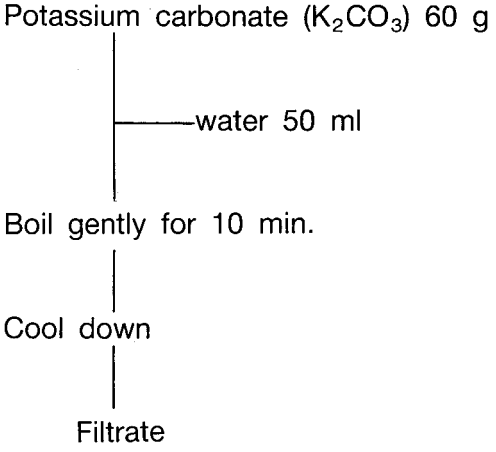
a) Inner ring solution



b) 0.02N-HCl (Accurate)

Dilute 20 ml of 1N-HCl with water and make the volume to 1 litre.

c) Saturated potassium carbonate



d) 50% saturated potassium carbonate

Dilute c) solution with water (1:1).

e) 10% TCA

Dissolve 100 g of trichloroacetic acid (CCl_3COOH) in 900 ml of water.

f) 5% TCA

Dilute e) solution with water (1:1).

g) Sealing agent

Tragacanth gum 3 g

- water 30 ml
- glycerine 15 ml
- 50% saturated potassium carbonate 15 ml

Mix well.

ANNEX II. SCHEMATIC FORM FOR PREPARATION OF SAMPLE FOR TVB-N AND TMA

Fish muscle 2 g

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Mortar on ice

- 4% TCA 8 ml

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Homogenize

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Stand for 30 mins with occasional grinding

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Centrifuge 3000 rpm, 0-4°C × 10 mins.

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Supernatant for analyses

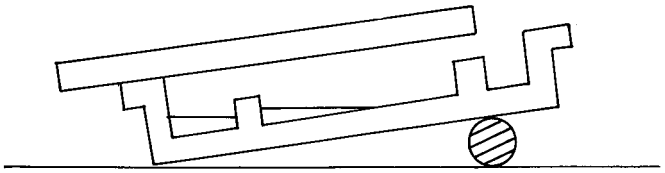
ANNEX III. PROCEDURE FOR TVB-N DETERMINATION

Sealing agent to Conway's unit

Inner solution 1 ml into inner ring

Sample extract 1 ml into outer ring

Slant the Conway's unit with cover



Saturated potassium carbonate
1 ml into outer ring

Close the unit using a band

Mix gently

Stand for 60 min. at 37°C

Titrate inner ring solution with 0.02N-HCl using a micro-burette

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

ANNEX IV. PROCEDURE FOR TMA-N DETERMINATION

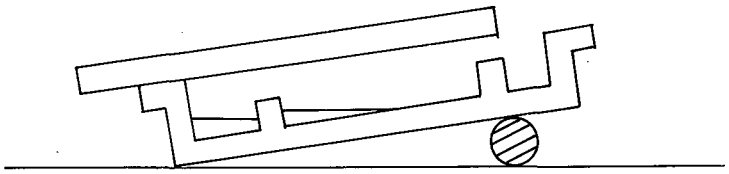
Sealing agent to Conway's unit

Inner solution 1 ml into inner ring

Sample extract 1 ml into outer ring

10% formaldehyde 1 ml into outer ring

Slant the Conway's unit with cover



Saturated potassium carbonate 1 ml into outer ring

Close the unit using a band

Mix gently

Stand for 60 min at 37°C

Titrate inner ring solution with 0.02N-HCl using a micro-burette

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

REFERENCES

Beatty, S.A. and N.E. Gibbons (1936)
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