DETERMINATION OF DMA-N BY DYER'S COLORIMETRIC METHOD USING COPPER DITHIOCARBAMATE

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INTRODUCTION

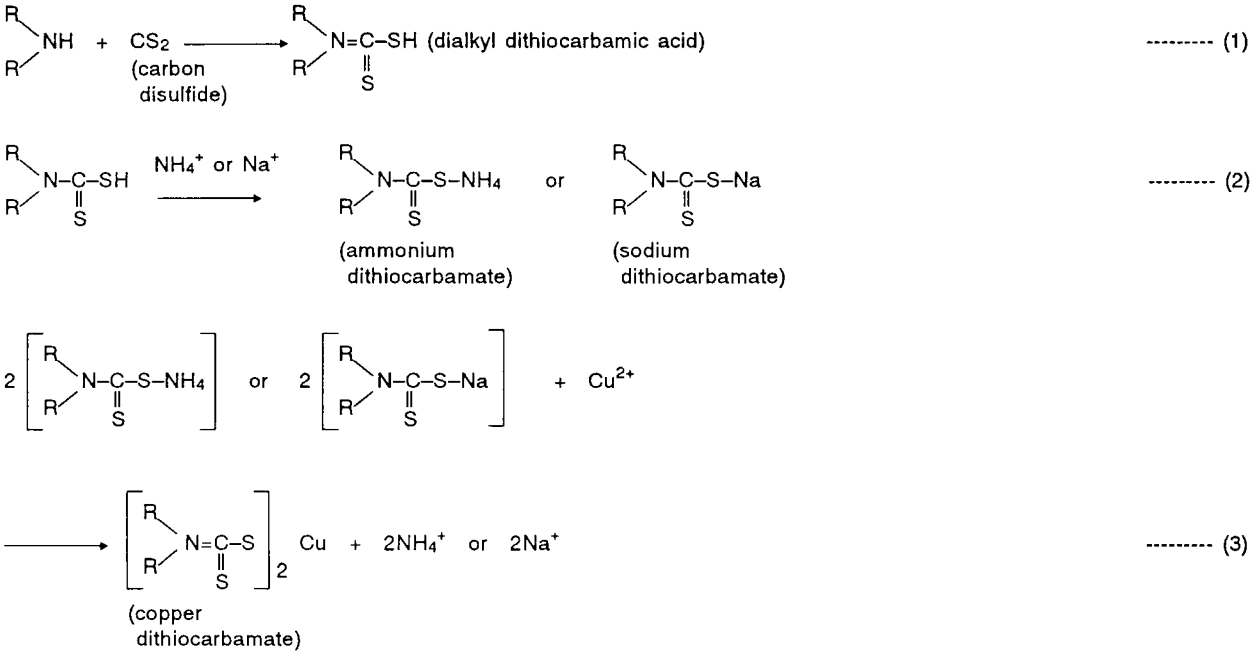
The precursor of dimethylamine (DMA) in fish meat is trimethylamine oxide (TMAO). In the gadoid species, TMAO present in ordinary muscle is decomposed to formadehyde (FA) and DMA simultaneously. This is usually attributed to endogenous enzymes. In the tropics, lizard fish is known to show a similar breakdown sequence. In fresh fish, and fish in the early stages of spoilage, the amounts of primary amines is low. The main secondary amine present is DMA. Hence measurement of DMA can be used as a spoilage indicator. However, at the later stages of spoilage, numerous other secondary amines are formed, and these will interfere with the results of the Dyer's colorimetric method.

In the laboratory determination of TMA-N and TMAO-N, the presence of DMA interferes and yields a higher value for the parameters measured. If the true amounts of TMA-N and TMAO-N are desired, the interference due to DMA must be discounted.

DMA and other secondary amines can react with nitrite salts to form dimethylnitrosoamine, a known carcinogen. Therefore, it is important to determine the amount of DMA present in fish and other food.

PRINCIPLE OF DYER'S COLORIMETRIC METHOD

Volatile secondary amines such as dimethylamine, di-n-propylamine etc. react with carbon disulfide to form dialkyl-dithiocarbamic acid (Equation 1). This dialky-dithiocarbamic acid react with NH_4^+ or Na^+ to form dithiocarbamate (Equation 2). Dialkyl-dithiocarbamate chelates with Cu^{2+} to form a yellow complex, Cu-dialkyl-dithiocarbamate (Equation 3).



I REAGENTS

All reagents should be of GR grade.

a) 5% (v/v) carbon disulfide-toluene (CS_2) solution
Mix 5 ml of carbon disulfide with 95 ml of toluene.

b) Copper-ammonium reagent

Dissolve 25 g of ammonium acetate and 0.2 g of cupric sulfate in 30 ml of distilled water, and mix this solution with 25 ml of 40% NaOH. To this add 20 ml of conc. ammonia (s.g. 0.88-0.90) and mix well, then make up to 100 ml with distilled water.

c) 30% acetic acid.

d) Anhydrous sodium sulfate.

e) DMA standard stock solution.

Take 60.0 mg of DMA-HCl salt into 100 ml volumetric flask and make up with distilled water. This solution contains about 0.1 mg DMA-N/ml.

f) DMA standard working solution

Take 10 ml of DMA stock solution into 100 ml of volumetric flask and make up with 2% TCA solution. This solution contains about 10 ug DMA-N/ml.

g) 25% TCA, 2% TCA

II PROCEDURE

A. SAMPLE PREPARATION

1. Take 5 g of sample in a mortar and grind well.
2. Wash the sample into a 100 ml volumetric flask with about 50 ml of distilled water.
3. Stand for 10 min after stirring well.
4. Add 8 ml of 25% TCA and mix well.
5. Make up to 100 ml with distilled water and mix well.
6. Stand for 30 min at ambient temperature.
7. Filter the solution with filter paper (Whatman No. 41).

B. DETERMINATION OF DMA

The following procedure should be done in a fume cupboard.

1. Take 5 ml of the filtrate in a test tube with stopper.
2. Add 1 ml of copper-ammonium reagent and mix.
3. Add 10 ml of 5% CS_2-toluene solution and then stopper the test tube.
4. Stand for 2 min in 50°C water bath.
5. Shake for 1 min using shaker to bring the colour complex from water layer to solvent layer.
6. Add 1 ml of 30% acetic acid to be added while tube is warm.
7. Shake for 20-30 sec.
8. Stand for 10 min at ambient temperature.
9. Transfer the toluene layer to another tube containing about 0.5 g of anhydrous Na_2SO_4 after the toluene layer becomes clear.
10. Measure the absorbance at 440 nm.
11. Repeat the procedure with 5 ml of 2% TCA as blank.

C STANDARDISATION OF DMA STANDARD SOLUTION

Principle:

$$DMA.HCl \xrightarrow[\Delta,\ \text{steam}]{NaOH} DMA\uparrow + NaCl + H_2O \qquad (1)$$

$$DMA + H_3BO_3 \longrightarrow DMA.H_3BO_3 \qquad (2)$$

$$DMA.H_3BO_3 + H_2SO_4 \longrightarrow DMA.H_2SO_4 + H_3BO_3 \qquad (3)$$

1. Take 10 ml DMA stock solution (DMA.HCl) into distillation tube. Add 20 ml distilled water and 6 ml 10% NaOH.
2. Steam distill to vaporise the DMA, and absorb into 20 ml of 4% H_3BO_3.
3. Titrate $DMA.H_3BO_3$ solution with 0.05N H_2SO_4 using methyl red-bromocresol green mixed indicator.

4. Calculate the factor of DMA.HCl standard solution as:

$$\text{factor, f} = \frac{[(\text{Vol. of sample titration}) - (\text{Vol. of blank titration})] \times (\text{Mol. wt. of N equivalent}) \times (\text{Normality of } H_2SO_4)}{\text{Volume of DMA.HCl used}}$$

$$= \frac{(V_a - V_b) \times 14.00 \times 0.05}{10}$$

D. PREPARATION OF CALIBRATION CURVE

1. Take 0.4, 0.8, 1.2, and 1.6 and 2.0 ml of DMA standard working solution into the test tube (volume about 40 ml), and add 4.6, 4.2, 3.8, 3.4 and 3.0 ml of 2% TCA solution, respectively. These solutions contain 4, 8, 12, 16 and 20 ug DMA-N, respectively.

2. Repeat the procedure for determination of DMA.

E. CALCULATION OF DMA-N CONTENT OF SAMPLES

1. Obtain the amount of DMA-N (A ug) contained in 5 ml of sample solution from the calibration curve.

2. DMA-N (mg/100 g)

$$\text{DMA-N (mg/100 g)} = \frac{(\text{ug DMA-N converted to mg}) \times (\text{Make up volume}) \times (100\,\text{g meat}) \times f \times d}{(\text{Wt. of sample}) \times (\text{Volume of sample})}$$

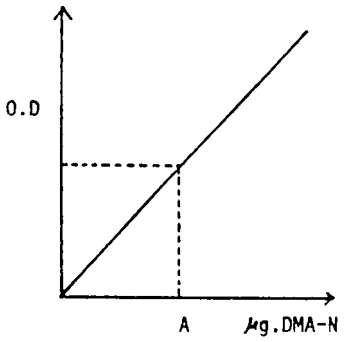
$$= \frac{A}{1000} \times \frac{100 \times 100 \times f \times d}{W \times 5}$$

where W = weight of meat

f = factor of DMA standard solution

d = dilution factor (if any)

A = readout from calibration curve



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Dyer W.J. and Y.A. Mounsey (1945). Amines in fish muscle. II. Development of trimethylamine and other amines. J. Fish. Res. Bd. Can. 6(5):359-367.