DETERMINATION OF FORMALDEHYDE IN FISH MEAT USING NASH'S REAGENT

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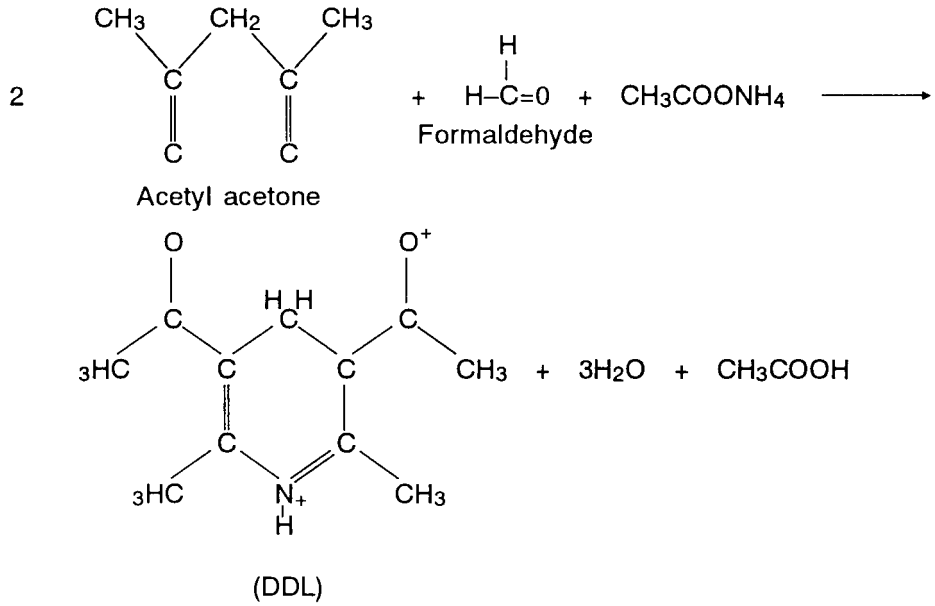
INTRODUCTION

It has been postulated that the enzymatic degradation of trimethylamine oxide (TMAO) results in the simultaneous formation of dimethylamine (DMA) and formaldehyde (FA). This phenomenon had been reported to have a correlation of 0.89. (Amano et al, 1963). FA and DMA formation occurs widely in the gadoid species. In the tropical area, lizard fish (Saurida sp) also exhibits this trend.

Formaldehyde reacts quickly with muscle tissues, causing protein denaturation. Formation of FA is accelerated by freezing.

In this method of FA determination, FA is reacted with an ammonium salt and acetylacetone under neutral conditions to form diacetyldihydrolutidine (DDL). DDL is a yellow compound with maximum absorbance at 412 nm.

MOLECULAR FORMULA AND REACTION



(DDL)

I REAGENTS

a) **Acetylacetone reagent (Nash's reagent)**

Dissolve 150 g of ammonium acetate, 3 ml of acetic acid and 2 ml of acetylacetone in distilled water and make up to 1 litre.

b) **Formaldehyde standard stock solution (1,000 ppm)**

Pipette 0.3 ml of 35% formaldehyde and make up to 100 ml with distilled water to get an approximately 1,000 ppm solution. This aqueous solution is stable for several months.

c) **Formaldehyde standard stock solution, working solution (10 ppm)**

Dilute the stock solution 100 times as follows:

Pipette 10 ml of the stock solution (approximately 1,000 ppm) and make up to 100 ml with distilled water to get approx. 100 ppm solution. Ten ml of 100 ppm solution is diluted 10 times with distilled water in the volumetric flask. This final dilute gives approx. 10 ppm solution of formaldehyde. This dilute is not stable, so, it is necessary to be renewed in each series of determination.

d) **0.1N Sodium thiosulphate standard solution**

Dissolve 25 g of $Na_2S_2O_3.5H_2O$ in distilled water which is cooled after boiling, and make up to 1 litre. Standardize after standing 1 to 2 days by the procedure described in the determination of peroxide value (C-5).

e) **Sodium bisulfite solution (approximately 0.1N)**

Dissolve 5.2 of $NaHSO_3$ in distilled water and make up to 1 litre.

f) **Iodine solution (approximately 0.1N)**

Dissolve 12.7 g of I_2 and 40 g of KI in 25 ml of distilled water and make up to 1 litre.

g) **1.5% Starch solution**

Weigh 1.5 g of starch and add 100 ml of distilled water, then boil the solution for 30 sec.

STANDARDISATION OF FORMALDEHYDE SOLUTION

1. Pipette 5 ml of distilled water (blank solution) into a 200-300 ml conical flask and add 50 ml of distilled water.

2. Add 10 ml of 0.1N sodium bisulfite solution, and let it stand for about 30 min with occasional shaking.

3. Titrate the blank with iodine solution until the colour turns brown. Note the volume (A ml) of the iodine solution used.

4. Then titrate against sodium thiosulphate solution using 1 ml of starch as indicator until the solution turns colourless. Note the volume of sodium thiosulphate used.

5. Pipette 5 ml of formaldehyde solution (approximately 1,000 ppm) into a 200-300 ml conical flask, and add 50 ml of distilled water.

6. Add 10 ml of 0.1N sodium bisulfite solution and let it stand for 30 min with occasional shaking.

7. Add the known amount (A ml) of iodine solution, swirl and titrate against sodium thiosulphate solution using 1 ml of starch as indicator until the solution turns colourless. Note the volume of sodium thiosulphate used.

The specific gravity of 35% formaldehyde at 20°C is 1.08. The molarity of the 1000 ppm solution is 0.032375. Since 1 mole of formaldehyde is equivalent to 2 moles of sodium thiosulphate, using $N_1 V_1 = N_2 V_2$,

N_1, Normality of formaldehyde = factor x Molarity x equivalent

= f x M x e

= f x 0.032375 x 2

V_1 = Volume of formaldehyde = 5 ml

N_2 = Normality of $Na_2S_2O_3$ = 0.1

V_2 = Volume of $Na_2S_2O_3$

= (Vol titrated in sample) – (Vol titrated in blank).

= $V_S - V_B$

$(f \times M \times e) \times (5) = (0.1) \times (V_S - V_B)$

Therefore,

$$f = \frac{0.1 \times (V_S - V_B)}{5 \times M \times e}$$

$$= \frac{0.1 \times (V_S - V_B)}{5 \times 0.032375 \times 2}$$

II APPARATUS AND INSTRUMENTS

Hitachi Spectrophotometer (= 412 nm)
Beckman Model 3560 digital pH meter
Yamato ultra disperser
beakers (50 ml)
long test-tubes (15 ml)
burette (25 ml)
Pipetman micropipette (max vol = 1 ml)
filter paper (Whatman No. 41, ø 15 cm)

III PROCEDURE

A. SAMPLE PREPARATION

1. Weigh 5 g of minced meat accurately in a 30-50 ml beaker.

2. Add 20 ml of 5% TCA solution and homogenize well with homogenizer.

3. Stand in an ambient temperature for 30 min.

4. Filter the supernatent with filter paper, Whatman No. 41.

5. Add 10 ml of 5% TCA solution to the residue, homogenize again, then filter.

6. Neutralize the combined filtrate to pH 6.0-6.5 by using pH meter with 1N or 0.1N KOH dropwise, and make up to 50 ml with distilled water.

B. DETERMINATION OF FORMALDEHYDE

1. Take 3 ml of the neutralized filtrate in a test tube, add 3 ml of the acetylacetone reagent and mix well.

2. Stand in water bath (60°C) for 15 min.

3. Cool the solution in running water.

4. Measure the absorbance of the solution against the blank solution at 412 nm (Blank solution contains distilled water instead of the neutralized filtrate).

C. PREPARATION OF CALIBRATION CURVE

1. Pipette 0, 0.3, 0.6, 1.2 and 2.4 ml of 10 ppm formaldehyde standard working solution into test tube using micro-pipette. These solutions contains 0, 3, 6, and 12 and 24 ug of formaldehyde, respectively.

2. Add 3, 2.6, 2.4, 1.8 and 0.6 ml of distilled water, respectively, then add 3 ml of the acetylacetone reagent.

3. Continue as in the procedure B. 2. to 4. after mixing well.

IV CALCULATION OF FORMALDEHYDE CONTENT

$$\text{Formaldehyde (ug/g)} = \frac{A}{\text{(Vol. of filtrate used)}} \times \frac{\text{(Total make up vol. of filtrate)} \times f}{\text{(Weight of sample)}}$$

where A = Reading from calibration curve (ug)

f = factor of formaldehyde of standard solution.

REFERENCES

Amano K., K. Yamada and M. Bito 1963A.
Detection and identification of formaldehyde in gadoid fish. Bull. Jap. Soc. Sci. Fish. 29(7): 695-701.

Amano K., K. Yamada and M. Bito 1963B,
Contents of formaldehyde and volatile amines in different tissues of gadoid fish. Bull. Jap. Soc. Sci. Fish. 29(9): 860-864.

Hebard C.E., J.F. George and R.E. Martin.
Occurence and significance of trimethylamine oxide and its derivatives in fish and shellfish. In: Martin R.E. et al (Editors) 1982. Chemistry and biochemistry of marine food products p: 164-165. Publ. AVI., Westport.