DETERMINATION OF AMMONIA (Colorimetric method)

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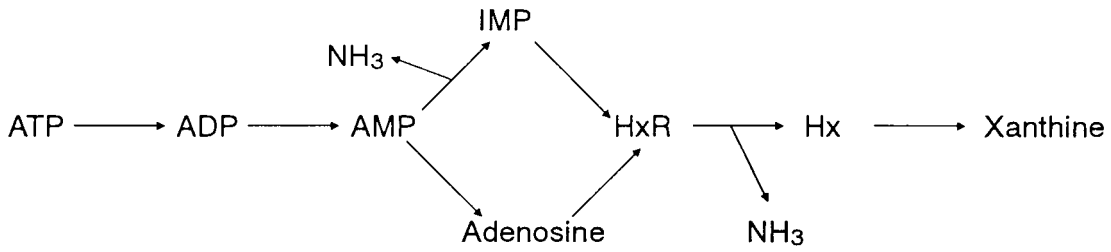
INTRODUCTION

Ammonia was assumed to come from the breakdown of nitrogen containing compounds in teleosts and, in the case of elasmobranchs, of urea. Non-protein nitrogen (NPN) constituents and creatine are utilized by Pseudomonads. The primary mode of utilization seems to be oxidative deamination resulting in the accumulation of ammonia and volatile fatty acids. In hake, lactic acid, NPN compounds such as trimethylamine oxide and amino acids are attacked by Pseudomonads to yield trimethylamine, dimethylamine, ammonia and volatile acids. In ice stored shrimps, ammonia can be produced by the action of both micro-organisms and tissue enzymes, adenosine deaminase and adenosine monophosphate deaminase. The degradation pathway is shown as follows :



Where,

ATP	=	adenosine triphosphate,
ADP	=	adenosine diphosphate,
AMP	=	adenosine monophosphate,
IMP	=	inosine monophosphate,
HxR	=	inosine
Hx	=	hypoxanthine
NH_3	=	ammonia

APPARATUS

1. Erlenmeyer flask with stoppers, 100, 250, 500 ml
2. Volumetric flasks, 25, 100, 1000 ml
3. Measuring cylinders, 10, 250 ml
4. Glass filter funnel, ø : 7 cm
5. Filter paper, Whatman No. 1
6. Transfer pipettes, 1, 2, 3, 4, 5, 20 ml
7. Graduated pipettes, 10 ml
8. Separatory flask, 125 ml
9. Timer
10. Glass wool
11. Spectrophotometer (wavelength = 680 nm)

REAGENTS

1. Sodium hydroxide solution, NaOH(1+1)

   Add 1 part distilled water to 1 part NaOH (reagent quality containing <5% Na_2CO_3) in flask. Swirl till solution is completely mixed. Close with rubber stopper. Set aside until Na_2CO_3 has settled, leaving perfectly clear liquid (ca. 10 days).

2. Bromine solution

   Dilute 10 ml NaOH(1+1) from (1) to ca. 100 ml with distilled water. Add 1.0 ml bromine and shake. Dilute to 200 ml with water. Prepare fresh daily.

3. Thymol solution (10% in alcohol)

   Weigh 2.5 g thymol and dissolve in alcohol and make up to 25 ml with alcohol. Prepare fresh daily.

4. Dilute sodium hydroxide solution

   Dilute 25 ml of NaOH(1+1) from (1) to 100 ml with distilled water.

5. Ammonia standard solution

   Dry 5 g of ammonium chloride, NH_4Cl at 100°C for 1 hour. Dissolve 0.314 g NH_4Cl in distilled water and make up to 100 ml. This gives a solution which contains 40 ug/ml. Transfer 4.0 ml to 100 ml volumetric flask, and dilute to volume with distilled water.

6. 2.5% phosphotungstic acid solution

   Dissolve 25 g phosphotungstic acid or tungstophosphoric acid hydrate in distilled water and make up to 1 L. This should not be kept for more than a week. Keep the solution in an amber bottle.

7. n-Butanol (GR)

8. Anhydrous sodium sulphate (Na_2SO_4)

PROCEDURE

Sample Preparation

1. Grind sample 3 times through food chopper, mixing after each grinding.

2. Place 20 g prepared sample in 500 ml Erlenmeyer flask.

3. Add 180 ml 2.5% phosphotungstic acid solution, shake vigorously for 2 min.

4. Filter through Whatman No. 1 or equivalent paper into 250 ml Erlenmeyer flask.

5. Pipette 2 ml filtrate (equivalent to 0.2 g sample) into 125 ml separator. Save remainder of filtrate.

6. To another separator, add 2.0 ml 2.5% phosphotungstic acid solution as blank.

7. To each separator add 8.0 ml distilled water.

8. Then in **immediate** succession add :

   a) 1.0 ml dilute NaOH solution (Reagent 4), swirl to mix.

   b) 2.0 ml thymol solution (Reagent 3), swirl to mix

   c) 5.0 ml bromine solution (Reagent 2) in ca. 30 small additions, swirling vigorously after each addition.

9. Shake vigorously for 1 min. Let stand for 20 min.

10. With series of samples or standards, complete reagent additions in sequence on each separator before proceeding to next.

11. To each separator add 20.0 ml n-butanol and shake vigorously for 1 min. Let stand for 20 min.

12. Drain aqueous layer and pass n-butanol layer through ca. 30 g anhydrous Na_2SO_4 glass funnel plugged with glass wool into 100 ml Erlenmeyer flask.

13. Measure absorbance of solution at wavelength maximum ca. 680 nm in 1 cm cell against blank as reference.

    If absorbance is higher than that of highest ammonia standard, quantitatively dilute reserved filtrate with 2.5% phosphotungstic acid solution so that 2.0 ml diluted solution will produce absorbance below this level.

14. Preparation of standard curve

a) Pipette 0, 1, 2, 3, 4, and 5 ml standard ammonia solution (Reagent 5) into 125 ml separators.

b) Add 2.0 ml 2.5% phosphotungstic acid solution.

c) Dilute up to a total of 10 ml with distilled water.

d) Proceed as in Procedure Step 8.

e) Using 0 solution as reference, measure absorbance of each standard at a wavelength maximum of ca. 680 nm.

f) Prepare standard curve.

REFERENCE

Martin, R.E., Flick, G.J., Hebard, C.E. and Ward, D.R. (1982). 1st Ed. Chemistry & Biochemistry of Marine Food Products. p 28, 31, 323 - 324.

Horwitz, W. (1980). Official Methods of Analysis of the Association of Official Analytical Chemists. 13th Ed. p 289.