OIV-MA-AS323-02B Total nitrogen

Type IV method

1. Principle

The sample is wet ashed using sulfuric acid in the presence of a catalyst. The ammonia liberated by sodium hydroxide is determined titrimetrically.

2. Apparatus

2.1. Digestion apparatus

300 mL Kjeldahl flask. Place on a metal heating mantle. Appropriate stand to hold this apparatus, the neck bent at 45 degrees.

2.2. Distillation apparatus

1 liter round bottomed flask, fitted with a small rectifying column 30 cm long by 2.5 cm diameter or any other equivalent apparatus. The vapor emitted from the end of this apparatus enters into the top part of the cylindrical condenser, held vertically, of 30 cm length and 1 cm internal diameter. The condensed liquid is brought to the receiving conical flask by a drawn□out tube placed at the bottom – alternatively one can use a steam distillation apparatus such as described in *Volatile Acidity*, or any other apparatus relating to the test described in paragraph "*Blank tests or sample tests*".

3. Reagents

3.1. Sulfuric acid free of ammonia (ρ_{20} = 1.83 □ 1.84g/mL)

3.2. Benzoic acid

3.3. Catalyst:

Copper sulfate, $CuSO_4$	10 g
Potassium sulfate, K_2SO4	100 g

3.4. 30%Sodium hydroxide solution. Sodium hydroxide (ρ_{20} =1.33 g/mL) diluted 30% (*m/m*).

3.5. 0.1 M Hydrochloric acid solution

3.6. Indicator:

Methyl red	100 mg

Methylene blue	50 mg
Ethanol (50%)	100 mL

3.7. Boric acid solution:

Boric acid	40 g
Water to	1000 mL

This solution will become pink by adding 5 drops of methyl red and 0.1 mL or more 0.1 M hydrochloric acid solution.

3.8. Ammonium sulfate solution:

Ammonium sulfate $(NH_4)_2SO_4$	6.608 G
Water to	1000 mL

3.9. Tryptophan, $C_{11}H_{12}O_2N_2$, (this substance contains in theory 13.72 g of nitrogen per 100 g)

4. Procedure

Place in the 300 mL Kjeldahl flask (2.1), 25 mL of wine, 2 g benzoic acid (3.2) and 10 mL sulfuric acid (3.1). Add 2 to 3 g of catalyst. With the flask placed on a metal disc mantle (2.1) and with the neck inclined at 45 degrees, heat until a clear color is obtained. Then heat for another 3 minutes.

After cooling, carefully transfer the contents of the Kjeldahl flask to a 1 liter round bottomed flask containing 30 mL water. Rinse the Kjeldahl flask several times with water and add washings to the round-bottomed flask. Cool the flask; add 1 drop of 1% phenolphthalein solution and a sufficient quantity of 30% sodium hydroxide solution (3.4) to ensure the solution is alkaline (40 mL approximately) making sure to cool the flask constantly during this addition. Distil 200 - 250 mL into a flask containing 30 mL of 40 g/L boric acid solution.

Titrate the distilled ammonia in the presence of 5 drops of indicator (3.6) using 0.1 M hydrochloric acid solution.

Note: A control trainer by vapor can be used as described in the Chapter on volatile acidity to obtain a quick ammonia distillation. In this case, successively place 40 to 45 ml of 30% sodium hydroxide liquor and 50 to 60 ml of previously diluted for 10

minutes contents of the Kjeldahl flask before introducing into the mixer.

5. Calculation

The total nitrogen, in g/L, contained in the wine is given by: 0.56 x *n* where *n* is the volume of 0.1 M hydrochloric acid.

6. Blank tests and sample tests

a) All distillation apparatus used to determine ammonia must satisfy the following tests:

Place in a distillation flask 40 □ 45 mL of sodium hydroxide solution, 50 mL water, 2 g benzoic acid, 5 g potassium sulfate and 10 mL sulfuric acid diluted to 50 mL. Distil 200 mL and collect the distillate in 30 mL of 40 g/L boric acid solution, to which 5 drops of indicator (3.6) are added. A change of color of the indicator must be obtained by adding 0.1 mL of 0.1 M hydrochloric acid solution.

b) Under similar conditions distill 10 mL of 0.1 M ammonium sulfate solution. In this case, between 10.0 and 10.1 mL of 0.1 M hydrochloric acid solution, must be used to change the color of the indicator.

c) The complete method (wet ashing and distillation) is checked using 200 mg tryptophan as the initial sample. Between 19.5 to 19.7 mL of 0.1 M hydrochloric acid must be used to obtain the change of color.