**AGROINDUSTRIAL STRENGTHENING OF QUINOA PROJECT THROUGH THE SCALING OF PROTOTYPES IN RELEVANT ENVIRONMENTS FOR THE INDUSTRY IN THE DEPARTMENT OF CAUCA**

**NITROGEN DETERMINATION**

**Guide Code: 003**

**INVESTIGATION GROUP:CYTBIA; ASUBAGROIN; GIPA.**

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1. **AIM**

This instruction is used to quantitatively determine the nitrogen content by the kjeldahl method, in a sample of animal or vegetable origin, according to the AOAC 968.06 method.

1. **BASIS**

The Kjeldahl method for determining nitrogen, introduced a century ago, is today the most widely used method for determining protein in organic materials, it has also found application in the determination of nitrogen in inorganic materials. The reason for its great acceptance and its official status is its high reliability and knowledge acquired through the years. Originally the method was heavy and long.

However, Raypa's DNP system has been developed to cover all efficiency requirements and reduce energy, reagent and time costs.

The nitrogen content, which is expressed as total nitrogen or crude protein, is always determined by liquid combustion in which nitrogen is first converted to ammonium sulfate and finally to ammonia, the ammonia is distilled, collected in boric acid and titrated. with a standard acid solution. This method, devised by J. Kjeldahl in 1883, has undergone numerous modifications, not in its fundamentals, but in what refers to the catalysts applied to speed up or make digestion more complete. In general, it consists of:

* Oxidation of the sample with H2SO4 and a catalyst, during which organic matter is destroyed and nitrogen is converted to ammonium hydrogen sulphate.
* Decomposition of ammonium acid sulphate by means of excess base or strong alkali to liberate ammonia, which is collected by distillation over boric acid.
* Titration of the ammonium borate forming, with HCL standard solution, using the end point of the mixture with Tashiro indicator as indicators.
* We multiply the amount of nitrogen by a factor depending on the raw material to be analysed.

Organic matter + H2SO4 450 °C-Catalyst CO2 + H2O + (NH4)2SO4

(NH4)2SO4+2NaOH Na2SO4 + 2H2O + 2NH3

NH3 + H3BO3 NH4H2BO3

NH4H2BO3 + 2HCl NH4Cl+H3BO3

1. **MATERIALS**

|  |  |
| --- | --- |
| **MATERIAL** | **AMOUNT** |
| Erlenmeyer | 4 |
| kjeldahl tubes | 7 |
| 10 mL pipettes | 1 |
| 25 mL burette | 1 |
| 50 mL measuring cylinder | 1 |
| beaker | 2 |
| Burette clamp | 1 |
| dropper | 1 |
| Spatula | 1 |
| Glass clock | 1 |
| Mortar | 1 |

1. **REAGENTS**

|  |  |
| --- | --- |
| **REAGENT** | **AMOUNT** |
| concentrated sulfuric acid | 60 mL |
| Kjeldahl catalyst | 6g |
| Sodium hydroxide 32% (w/v) | 500mL |
| Tashiro indicator | 5mL |
| Boric acid 3% (p/v) | 600 mL |
| Hydrochloric acid 0.1N | 100ml |
| Sodium hydroxide 20% (w/v) | 1000mL |

1. **TEAMS**

|  |  |
| --- | --- |
| **TEAM** | **AMOUNT** |
| kjeldahl digester | 1 |
| Distiller | 1 |
| scrubber | 1 |
| Balance | 1 |

1. **PROCESS**

The determination of nitrogen must be reported on a dry basis, for this the humidity must be previously determined.

* 1. **Preparation of sample**

The sample for the determination of nitrogen must be previously dehumidified and defatted.

* 1. **Reagent preparation**
     1. Tashiro indicator solution: Mix 25 mL of 0.05% alcoholic methylene blue solution and 25 mL of 0.1% methyl red solution (0.2 g of methyl red and 0.1 g of blue). of methylene in 100 mL of ethanol).
     2. Boric acid solution at 3% (w/v): dissolve 30 grams of H3BO3 in hot distilled water, cool and make up to 1000 mL.
     3. 0.1 N hydrochloric acid: 8.0 mL of HCl (37%-1.19 g/mL) in one liter of distilled water.
     4. Sodium hydroxide solution 32% (w/v): weigh 32 g of industrial sodium hydroxide, dilute in distilled water and make up to a 100 mL flask.
     5. Sodium hydroxide solution 20% (w/v): weigh 20 g of industrial sodium hydroxide, dilute with distilled water, and measure in a 100 mL flask.
  2. **Process**
     1. On nitrogen-free paper (tracing sheet) weigh 0.2 to 0.8 g of sample, plus 1 g of kjeldahl catalyst, transfer to digestion tubes.
     2. Add 10 mL of sulfuric acid.
     3. Start heating gently until no foaming or spattering is observed.
     4. Digest the samples until they are completely clear and translucent, free of organic matter. In the case of samples of animal origin, in the Raypa MBC-6/N equipment, the programming on the digester control panel can be found in the following table:

|  |  |  |
| --- | --- | --- |
| **temperature ramp** | **Temperature (°C)** | **Time (minutes)** |
| one | 125 | 30 |
| 2 | 270 | 30 |
| 3 | 400 | 140 |



neutralization unit

digestion tubes

Control Panel

* + 1. Cool to room temperature and take the tube with the sample to the Raypa brand distillation unit, select the program that indicates protein determination.
    2. Place each tube in distillation equipment, with its respective Erlenmeyer, lThe distillation must be regulated in the program of the Raypa brand distiller in order to produce 200 mL of distillate in the established time.



In the programming panel of the equipment, the following data must be recorded that correspond to those optimized for the determination, in terms of percentage of steam, the amount of boric acid, water, sodium hydroxide and the distillation time so that 200 are collected. mL of the distillate, the delay time (DELAY) is given for the addition of the T-Shiro indicator.

|  |  |  |
| --- | --- | --- |
| **H3BO3** | 30 mL | STEAM: 70% |
| **H2O** | 75 mL | VACUUM: OFF |
| **NaOH** | 50ml |
| **DELAY** | 00´15” |
| **DESTINY** | 06:30” |

* + 1. After each sample distillation, the washing program is selected and 2 consecutive washes are carried out.
    2. Each sample is titrated with a burette, which contains 0.1 N HCl.
  1. **Calculations and expression of results**

Protein percentage will be calculated according to the following table:

|  |  |
| --- | --- |
| **RAW MATERIAL** | **CONVERSION RATIO** |
| meat and other | %N\*6.25 |
| Milk | %N\*6.38 |
| Corn | %N\*5.65 |
| Wheat | %N\*5.33 |
| Rice | %N\*5.17 |

***NOTE: T****All procedures must be performed in triplicate, a reagent blank must be performed throughout the process to eliminate interference from the materials used.*

1. **DISPOSAL OF CHEMICAL AND/OR BIOLOGICAL WASTE.**

Residual digestion solution, add heavy metal container number 6.

Neutralization solution, add container of acids and bases, number 9.

**BIBLIOGRAPHY**

AOAC 968.06. 19th ed.Gaithersburg, MD: The association, 2012. Determination of the nitrogen content. In: Official Methods of Analysis.

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