INSTITUTE OF CHEMICAL TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

ANALYSIS OF FOOD AND NATURAL PRODUCTS

LABORATORY EXERCISE

Determination of total nitrogen in food and crude protein calculation
(Kjeldahl method)

Responsible person: Assoc.Prof. Ing.Kateřina Riddellová, Ph.D.

CONTENT

Required knowledge

- proteins – definition of crude, true and digestible proteins. Principle of ammonia and total nitrogen determination in food using the Kjeldahl method
- principle of wet ashing
- titrimetric methods: principle of volumetric analysis, basic notions, related calculations
- solutions – basic calculations

Evaluative criteria

- proved theoretical knowledge
- quality of practical work execution
- compliance with laboratory work rules (safety, order)
- protocol – integrity of experimental data, results calculation and discussion

Laboratory exercise content

A. **Total nitrogen determination according to the Kjeldahl method using the Kjeltec system 1002** (based on ČSN ISO 1871; adapted for teaching purposes and for the Kjeltec instrument)

B. **Ammonia determination according to the Kjeldahl method using the Parnas-Wagner apparatus**

Specification – nitrogen determination using the Kjeltec system:

- Each student in a pair conducts one complete analysis of the same flour sample, including digestion. Each student in a pair states both results in his/her individual protocol.
- Analysis of diluted protein hydrolysate prepared for ammonia determination (see below) is as well performed.

Specification – nitrogen determination using the Parnas-Wagner appartaus:

- Each student in a pair (or threesome) analyses once the same sample of (diluted) protein hydrolysate.
- The same sample is also analysed using the Kjetltec system.
- The results acquired by both methods are stated in the protocol.

Laboratory exercise schedule

TASK	DURATION (min)	Note
Introduction and examining	30 min	
Weighing and preparation of samples for digestion	30 min	
Digestion	90 - 120 min	
Introduction to the Parnas-Wagner apparatus	20 min	After starting the digestion
Performing ammonia determination using the Parnas –Wagner apparatus	100 min	Simultaneously with the digestion
Introduction to the Kjeltec system	20 min	
Performing nitrogen determination using the Kjeltec system	60 - 100 min	
Cleaning	20 - 30 min	

A. TOTAL NITROGEN DETERMINATION ACCORDING TO THE KJELDAHL METHOD USING THE KJELTEC SYSTEM 1002

1. SCOPE

The method is applicable to the determination of nitrogen occurring in the trinegative state in food and raw materials. It does not apply to N-N and N-O linkages (e.g. azides, nitrates, nitrites, nitro groups, etc.).

2. BASIC PRINCIPLE

The method consists of three steps: 1) DIGESTION of the sample in sulphuric acid with a catalyst. The nitrogen contained in the sample is converted to ammonia; ammonium sulphate being formed. 2) DISTILLATION of ammonia released from ammonium sulphate by addition of an excess of sodium hydroxide; ammonia being trapped in a trapping solution (sulphuric acid). 3) BACK-TITRATION of the excess of the trapping solution.

3. EQUIPMENT, MATERIALS AND SPECIAL LABORATORY GLASS

- analytical scales
- digestion unit Tecator 2006
- stand for digestion tubes (6 positions)
- digestion tubes (250 mL)
- vapour exhauster with Teflon sealing connected to a water vacuum aspirator - Tecator Exhaust DS6
- distillation unit Kjeltec 1002 equipped with a canister containing 30 % (w/w) solution of sodium hydroxide
- common laboratory glassware

4. CHEMICALS AND SOLUTIONS

- distilled water
- sulphuric acid for digestion, concentrated, 95 – 98 %
- mixed catalyst digestion tablets - KJELTABS S/3.5 (3.5 g K_2SO_4 + 0.0035 g Se)

- sodium or potassium hydroxide (to release ammonia from mineralizate) - 45 % (w/w) solution
- Tashiro indicator
- sulphuric acid (trapping solution), concentration c= 0.05 mol/L
- sodium hydroxide (titrant), concentration c= 0.1 mol/L

All chemicals of p.a. grade.

5. PROCEDURE

5.1 Digestion

Place 2 digestion tablets into a digestion tube. Weigh approximately 1 g of sample (to a precision of 2 decimal places) using a weighing boat. Quantitatively transfer the sample into the digestion tube using 12 mL of concentrated sulphuric acid.

Warning: ***Check the bottom of the digestion tubes for star cracks prior to adding the sample and reagents. Only handle the concentrated sulphuric acid in a dedicated fume hood; wearing protective gloves and a face shield.*** *First pour the acid into a beaker. Then measure 12 mL from the beaker into a cylinder. Transfer the sample to the digestion tube such that the walls stay clean. Rinse off any remains of the sample using sulphuric acid from the cylinder.*

After shaking it in a circular fashion, place the tube in a digestion stand. Attach a vapour exhauster, switch a water vacuum aspirator on and set the recommended digestion temperature (i.e. 420 ^{o}C). After the required temperature is reached (approx. 15 minutes), digest the sample for 40 minutes. Switch the digestion unit off and let the stand with the digestion tubes cool down in a separate place (approx. 20 minutes).

Warning: *Only the instructor may put the digestion tubes in the digestion unit, or take them out. Only the instructor may switch the digestion unit on/off.*

Note: Digestion was shortened for the purpose of this exercise. To enable the digestion of resistant compounds (Lysin, Tyrosin) an additional 60 minutes of digestion would be necessary after clearing up the tube´s contents.

5.2 Distillation using the distillation unit KJELTEC 1002

5.2.1 Preparation of the distillation unit

(Plug in the unit and press the main (green) switch. After checking that the valve labelled "DRAIN", located at the back of the unit, is closed (handle parallel to the back), turn the water on.)

Place a clean digestion tube (half filled with distilled water) and an Erlenmayer flask into the distillation unit, and close the safety window. By pressing the handle labelled "STEAM", start the

steam generator up and let it run for 5 minutes to clean and preheat the whole unit. After 5 minutes stop steam generation by pulling the handle "STEAM" to the upper position. Open the safety window, remove the tube and the Erlenmayer flask, and discard their contents into the sink with cold water running.

Warning: ***Use heat protective gloves to manipulate digestion tubes – they are HOT!*** *Do not touch the Teflon tubing with your hand, but use the digestion tube to direct it into the holder.*
The distillation unit must be cleaned after distillation of each sample.

5.2.2 Sample distillation

Carefully dilute the cool digest by adding 30 mL of distilled water, and place the tube into the distillation unit.

Warning: *Be aware of the danger of handling concentrated sulphuric acid and of adding water into acid!!! The addition of water has to be done* ***in a dedicated fume hood while wearing protective gloves and a face shield!***

Place a titration (receiver) flask containing 25 mL sulphuric acid (c= 0.05 mol/L) and a few drops of Tashiro indicator into the unit, and raise the platform. Make sure that the end of the cooler is under the surface; if not, add a small amount of distilled water.

- close the safety window
- gently press the "ALKALI" handle all the way down to dispense 45 ml 45 % (w/w) sodium hydroxide solution
- open the "STEAM" valve by pulling the handle down
- set the timer to 3.5 minutes
- after hearing the signal, lower the platform with the receiver flask so that the end of the cooler is above the surface
- close the "STEAM" valve
- open the safety window, remove the tube and discard the content into the sink with cold water running
- the contents of the receiver flask is ready for titration
- clean the unit according to the procedure described in section 5.2.1

Warning: *Do not close the "STEAM" valve before lowering the receiver flask, otherwise the distillate is sucked into the cooler.*
Use heat protective gloves to manipulate digestion tubes – they are HOT! *Do not touch the Teflon tubing with your hand, but use the digestion tube to direct it into the holder.*
When removing the tube and the receiver flask use a washing bottle to rinse the Teflon tubing.

5.2.3 Final cleaning of the unit

Rinse the unit with distilled water and use a wet cloth to clean all parts (especially the rubber adapter) that were in contact with hydroxide or acid.

Remove the drain trough at the bottom of the unit and clean it with water. Wipe the unit to remove any spillage. Place an empty digestion tube and a receiver flask into position, and close the safety window.

5.2.4 Closing down

Close the water tap, switch off the power, and open the valve labelled "DRAIN" at the back of the unit. All the water in the expansion vessel and steam generator will flow out.

5.3 Titration

5.3.1 "Blank" titration

Blank titration is carried out to test the colour change of Tashiro indicator. Tashiro indicator is a mixture of methylen blue and methyl red, the colour of which changes (depending on pH) as follows:
acidic range: violet *neutral range: colourless, greyish tinge* *basic range: green*
The endpoint (equivalence point) is then identified by the decolorization of an originally violet solution. By adding one more drop of titrant the solution turns green (i.e. over-titrated).

Using a burette, add 10 mL of sulphuric acid (c = 0.05 mol/L) into a titration flask containing approx. 10 mL of water and a few drops of Tashiro indicator. Titrate this solution using sodium hydroxide (c = 0.1 mol/L), the volume of added titrant should be 10 mL.

5.3.2 Sample titration

Titrate the contents of the receiver flask (see 5.2.2) with sodium hydroxide solution (c = 0.1 mol/L) to the neutral endpoint. Record the volume of hydroxide required.

5.4 Calculation of results

The reactions involved in the whole procedure are summarised as follows:

Sample digestion
Organic N + $H_2SO_4 \rightarrow CO_2 + H_20 + (NH_4)_2SO_4$

Neutralisation of digestion mixture and release of ammonia
$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

Reaction of ammonia with trapping solution (H_2SO_4)
$2NH_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4$ $\mathbf{n_{NH3} = 2n_{H2SO4}}$

Back titration of the excess of trapping solution
$H_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O$ $\mathbf{n_{H2SO4} = ½\ n_{NaOH}}$

With respect to stoichiometry, and taking into account the concentration of titrant (NaOH, c = 0.1 mol/L) and trapping (H_2SO_4, c = 0.05 mol/L) solution, the volume of sodium hydroxide needed to titrate the sample (V_{NaOH}) corresponds to the excess of sulphuric acid in the trapping solution ($V_{ExH2SO4}$).

To calculate the amount of nitrogen in the sample it is necessary to determine the amount of sulphuric acid (trapping solution) that reacted with ammonia to form ammonium sulphate ($V_{React\ H2SO4}$), i.e. to subtract the excess of sulphuric acid from the total (original) amount of sulphuric acid in the trapping solution ($V_{TotH2SO4}$)*:*

$$\mathbf{V_{React\ H2SO4} = V_{TotH2SO4} - V_{ExH2SO4}} \quad \textbf{(ml)} \qquad \mathbf{V_{ExH2SO4} = V_{NaOH}}$$

The amount of nitrogen is then calculated according to the following equality:

1 mL of 0.05 mol/L H_2SO_4 …….corresponds to……..1.4 mg of nitrogen

The result should be expressed in g of nitrogen per 100 g of sample, and also in g of crude protein per 100 g of sample.

To calculate the crude protein, multiply the amount of nitrogen by factor F **(**$F_{flour} = 5.70$*)*

B. AMMONIA DETERMINATION ACCORDING TO THE KJELDAHL METHOD USING THE PARNAS-WAGNER APPARATUS

1. SCOPE

The method is applicable to the determination of ammonia in acidic protein hydrolysates.

2. BASIC PRINCIPLE

Ammonia, released from the sample by addition of an excess of sodium hydroxide, is distilled and trapped in a trapping solution (sulphuric acid). Back-titration of the excess of the trapping solution follows.

3. EQUIPMENT, MATERIALS AND SPECIAL LABORATORY GLASS

- analytical balance
- digestion tubes (250 mL)
- Parnas-Wagner distillation apparatus

4. CHEMICALS AND SOLUTIONS

- distilled water
- sodium or potassium hydroxide (to release ammonia from ammonium sulphate) - 35 % (w/w) solution
- Tashiro indicator
- sulphuric acid (trapping solution), concentration c= 0.05 mol/L
- sodium hydroxide (titrant), concentration c= 0.1 mol/L

All chemicals of p.a. grade.

5. PROCEDURE

5.1 Sample preparation

Weigh approximately 5 g of sample (to a precision of 2 decimal places) into a 50 mL volumetric flask. Make up the volume using distilled water.

5.2 Distillation

5.2.1 Distillation using the Parnas-Wagner apparatus

Preheat and rinse the apparatus with distilled water. Place a titration (receiver) flask containing 25 mL sulphuric acid (c= 0.05 mol/L) and a few drops of Tashiro indicator under the end of the cooler, make sure the cooler is under the surface; if not, add a small amount of distilled water. Using a pipette, transfer 10 mL of the diluted sample into the apparatus. After rinsing the funnel with distilled water add 20 – 30 mL 35 % (w/w) sodium hydroxide solution. Rinse the funnel using distilled water again. Measure 15 min from the first evolution of steam (bubbles going through the sample). Rinse the cooler end into the receiver flask using distilled water. The contents of the receiver flask is ready for titration. Remove the rest of the sample and rinse the apparatus with distilled water.

5.2.2 Distillation using the distillation unit KJELTEC 1002

Using a pipette, transfer 10 mL of the diluted sample into a digestion tube containing approx. 30 mL of distilled water. Further proceed according to the chapter 5.2.2 in Determination of nitrogen.

5.3 Titration – see determination of nitrogen

5.4 Calculation of results

The reactions involved in the whole procedure are summarised as follows:

Reaction of ammonia (released from sample) with trapping solution (H_2SO_4)

$2NH_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4$ $\quad$ $\mathbf{n_{NH3} = 2n_{H2SO4}}$

Back titration of the excess of trapping solution

$H_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O$ $\quad$ $\mathbf{n_{H2SO4} = ½\ n_{NaOH}}$

With respect to stoichiometry, and taking into account the concentration of titrant (NaOH, c = 0.1 mol/L) and trapping (H_2SO_4, c = 0.05 mol/L) solution, the volume of sodium hydroxide needed to titrate the sample (V_{NaOH}) corresponds to the excess of sulphuric acid in the trapping solution ($V_{ExH2SO4}$).

To calculate the amount of ammonia in the sample it is necessary to determine the amount of sulphuric acid (trapping solution) that reacted with ammonia to form ammonium sulphate ($V_{React\ H2SO4}$), i.e. to subtract the excess of sulphuric acid from the total (original) amount of sulphuric acid in the trapping solution ($V_{TotH2SO4}$)*:*

$$\mathbf{V_{React\ H2SO4} = V_{TotH2SO4} - V_{ExH2SO4}} \quad \textbf{(ml)} \qquad \mathbf{V_{ExH2SO4} = V_{NaOH}}$$

The amount of ammonia is then calculated according to the following equality:

1 mL of 0.05 mol/L H_2SO_4 …….corresponds to…….. 1.7 mg of ammonia

The result should be expressed in g of ammonia per 100 g of sample.