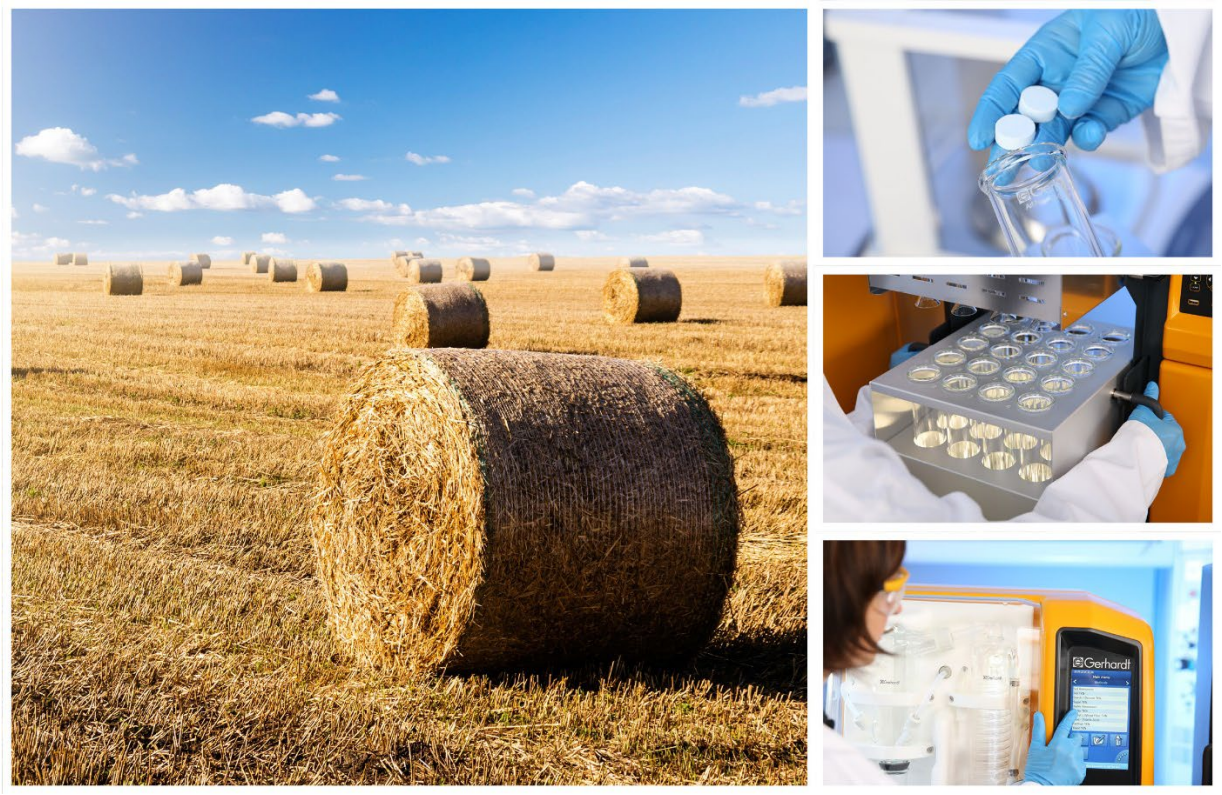
# Kjeldahl Application

## A.1.1.5. Nitrogen Determination in Grain and Grain Products

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## 1 Principle

The organic bound nitrogen of the sample is digested in concentrated sulphuric acid in the presence of a salt to increase the boiling point and converted into ammonium sulphate. The acidic digestion is alkalized by a caustic soda solution. The resulting ammonia is released and distilled by means of water steam into a boric acid receiver solution. The process is finalized with a titration with an acid solution with a known concentration. The nitrogen content is calculated by using the consumption of the titration solution and is converted to protein by means of the referring protein factor.

## 2 Methods

This application note is meant to be a guideline for the operation of your C. Gerhardt analysis system and has to be adapted to your sample matrix and the local circumstances in your laboratory.
The document is based on

- DIN EN ISO 20483 Cereals and pulses - determination of the nitrogen content and calculation of the crude protein content - Kjeldahl method, German version EN ISO 20483:2013
- Arbeitsgemeinschaft Getreideforschung, Standard-Methoden für Getreide, Mehl und Brot, Bestimmung des Proteins in Getreide und Getreideprodukten, ICC-Standard Nr. 105
- AOAC Official Method 979.09, Protein in Grain
- GAFTA 4:1, CRUDE PROTEIN – Kjeldahl Method, 2014.

## 3 Chemicals und material

Quality grade p. a.

3.1. Water: demineralized or distilled
3.2. Concentrated sulphuric acid $H_2SO_4$
3.3. Catalyst tablets KJELCAT Cu 5,0 g K2SO4 + 0,5 g CuSO4 + 5H2O (Art. 12-0328) or comparable
3.4. Caustic soda NaOH 32 %
3.5. Boric acid $H_3BO_3$ 2 %
3.6. Indicator solution M5 (Merck) or comparable
3.7. Standard solution: Hydrochloric acid c(HCl)= 0.1 mol/l or sulphuric acid c($H_2SO_4$) = 0.05 mol/l
3.8. Acetanilide
3.9. Sucrose, nitrogen-free
3.10. Ammonium sulphate, to be dried for at least 2 hours at a temperature of 102° C ± 2°C immediately before usage and subsequently to be kept in a desiccator for cooling to room temperature
3.11. Weighing paper WP250 (Art. 1004939) or paper weighing boats

## 4 Instruments

- Mechanical shredder e.g. grist mill or star mixer
- Analytical Balance (Accuracy 0.1 mg)
- Kjeldahl digestion system KJELDATHERM, TURBOTHERM or flask heater for Kjeldahl flasks with enlarged neck
- Fume Scrubber TURBOSOG, alternatively VACUSOG or water jet pump VAPODEST Steam distillation system, models 200 to 450 without titrator, titration has to be performed by means of a manual burette (class A, according to ISO 385), 50 ml nominal volume, with volume scale in 0.05 ml steps or a titrator, or instead of indicator solution with a pH meter with a combination electrode.
  The titration is performed automatically in case of VAPODEST 450 with external titrator or VAPODEST 500/500c with integrated titrator.

# 5 Procedure

## 5.1 Sample Preparation

Take a representative sample of at least 50 g. Grind the samples thoroughly to achieve that at least 90 % of the substance passes through a test sieve with mesh size 0.8 mm acc. to DIN EN ISO 20483 or mesh size 1 mm according to GAFTA 4.0.
Weigh the samples in weighing papers and place them into the digestion tubes.
Add the chemicals. Use sulphuric acid to wash down any sample residue which might remain at the glass walls.
Typical sample weights range from 1-2 g.

## 5.2 Digestion

| Chemicals | Amount per Sample |
|---|---|
| Sulphuric acid | 20 ml |
| KJELCAT | 2 |

### 5.2.1 Digestion with KJELDATHERM

For digestions with a KJELDATHERM system with 250 ml KJELDATHERM digestion tubes, we recommend the following program parameters:

| Phase | Step | hh:mm | Temp. [°C] | Power [%] | Lift | Suc | Cool Vent | Cool Water |
|---|---|---|---|---|---|---|---|---|
| Digestion | 1/2 | 01:30 | 410 | | | | | |
| Cooling | 2/2 | 00:30 | - | - | | | | |
| Done | | - | - | - | | | | |

If your digester does not have an automatic lift system, take out the insert rack after digestion manually and leave the samples for cooling.

**Tip**: Shorten the digestion time by placing the samples in a pre-heated digester.

### 5.2.2 Digestions with TURBOTHERM TTs

Using TURBOTHERM with 12 x 250 ml KJELDATHERM digestion tubes, we recommend the following program parameters:

| Note | Step | hh:mm | Power [%] | Suc |
|---|---|---|---|---|
| Heat-up of the system until boiling of the digestion solution | 1/3 | 00:15 | 100 | |
| After 20 – 30 minutes the digestion solution should be clear | 2/3 | 01:15 | 75 | |
| Cooling | 3/3 | 00:30 | 0 | |
| Done | | - | - | |

### 5.2.3 Digestions with TURBOTHERM TTs and foaming samples

For digestions with a TURBOTHERM system with 12 x 250 ml KJELDATHERM digestions tubes, we recommend the following program parameters:

| Note | Step | hh:mm | Power [%] | Suc |
|---|---|---|---|---|
| Heat-Up of the system until boiling of the digestion solution | 1/7 | 00:05 | 100 | ☑ |
| | 2/7 | 00:05 | 0 | ☑ |
| | 3/7 | 00:05 | 100 | ☑ |
| | 4/7 | 00:05 | 0 | ☑ |
| | 5/7 | 00:05 | 100 | ☑ |
| After 20 – 30 minutes the digestion solution should be clear | 6/7 | 01:30 | 75 | ☑ |
| Sample cooling | 7/7 | 00:30 | - | ☑ |
| Digestion done | | - | - | - |

Choose the method from the method library or program an older TURBOTHERM unit following the method „**Foaming Samples**“.

### 5.2.4 Digestions with a classic flask heater

For digestions with classic flask heaters in Kjeldahl flasks of 500 ml or 750 ml volume with enlarged neck, we recommend the following program parameters:

| time [min] | Power level | Note |
|---|---|---|
| 20 | 3 | Heating and evaporation until the digestion solution boils and white foams occur |
| 60 | 1,5 | After 20 – 30 minutes the digestion solution should be clear |
| 30 | - | Cooling down samples |

### 5.2.5 Digestions acc. to official norms (GAFTA 4.0, ISO 5983-1:2005)

For digestions acc. to official norms in a KJELDATHERM system with 250 ml KJELDATHERM digestion tubes, we recommend the following program parameters:

| Phase | Step | hh:mm | Temp. [°C] | Power [%] | Lift | Suc | Cool Vent | Cool Water |
|---|---|---|---|---|---|---|---|---|
| Digestion | 1/2 | 02:30 | 410 | | | ☑ | ☐ | ☐ |
| Cooling down | 2/2 | 00:30 | - | - | | ☑ | ☑ | ☐ |
| Digestion done | | - | - | - | | ☐ | ☐ | ☐ |

If your digester does not have a lift system, the insert rack has to be taken out manually after the digestion to allow the samples to cool down.

The digestion solution should be clear after approximately 30 minutes. If not all of the samples have turned clear, continue digesting another 30 to 40 minutes.

**Tip**: Shorten the digestion time by placing the samples in a pre-heated digester.

### 5.2.6 Suction of the digestion fumes

During the digestion, a fume scrubber (TURBOSOG or VACUSOG) must be activated. For the washing bottle we recommend to fill approx. 1.200 ml of caustic soda (concentration approx. 15 %). The suction power is adjusted correctly when no fumes come out of the tubes. You can check if the caustic soda is still usable by adding an indicator and checking the pH value.
Allow 30 minutes for cooling down after taking out the insert rack or after deactivating the heating. Leave the fume scrubber activated during this time.

**Tip**: You can shorten the cooling down time of your samples by half with a KJELDATHERM ECO KIT.

### 5.3 Distillation with VAPODEST

After cooling down the samples, a steam distillation is performed with the following program:

| | Method Food / Feed TKN | VAP 200 | VAP 300 | VAP 400 | VAP 450 | VAP 500 / 500c |
|---|---|---|---|---|---|---|
| $H_2O$ Addition | 100 ml | • | ✓ | ✓ | ✓ | ✓ |
| NaOH Addition | 80 ml | ✓ | ✓ | ✓ | ✓ | ✓ |
| Reaction time | 0 s | ✓ | ✓ | ✓ | ✓ | ✓ |
| Distillation time | 240 s | ✓ | ✓ | ✓ | ✓ | ✓ |
| Steam power | 100 % | ✓ | ✓ | ✓ | ✓ | ✓ |
| Sample suction | 30 s | - | ✓ | ✓ | ✓ | ✓ |
| $H_3BO_3$ Addition | 80 ml | • | • | ✓ | ✓ | ✓ |
| Suction receiver solution | 30 s | - | - | - | ✓ | ✓ |
| Titration | - | • | • | • | ✓ | ✓ |
| Calculation | - | • | • | • | • | ✓ |
| Reading pH value, fixed endpoint or automatic endpoint | - | - | - | - | ✓ | ✓ |
| Titration online | - | - | - | - | - | ✓ |

✓ = automatic • = manual - = not applicable

Choose the method from the method library or program an older VAPODEST unit following the method „Grain / Grain TKN“.

**Note:** If you use a different amount of sulfuric acid for digestion, also the addition of water and caustic soda during distillation has to be adjusted accordingly. A guideline for the proportions is: „1 part acid : 5 parts water : 4 parts caustic soda“ .

### 5.4 Titration

Add 3-4 drops of mixed indicator M5 to the receiver solution (3.6) and titrate with standard solution (3.7) until the colour changes from green to violet. If you determine the endpoint with a pH-meter or titrator, you do not have to add the mixed indicator M5.

### 5.5 Blank value

For blank value determination, perform the analysis (digestion + distillation + titration) just with the indicated chemicals and 1 g saccharose (3.9) instead of the sample. The chemical consumption has to be taken into account for the calculation.

### 5.6 Performance check

To check the analytical performance of your water steam distillation system, perform a distillation and titration of 0,120 g ammonium sulphate (3.10). The percentage of the recovered nitrogen must be between 99.0 and 100.0 % taking the purity of the standard solution into account. A recovery rate up to 101 % is still acceptable in sporadic cases.
To verify the method, perform the analysis with 0.2-0.3g acetanilide (3.8) in the presence of 1g sacarose (3.9) following this procedure. The recovery rate of nitrogen must be at least 99 %.

# 6 Evaluation

## 6.1 Nitrogen calculation in %

The nitrogen percentage can be calculated with the following equation:

$$\omega_{\%,N} = \frac{(V_1 - V_0) \times c_{eq,soll} \times t \times 1{,}4007}{m_{Sample}}$$

$\omega_i$ = Mass fraction of the sample [%]
$V_1$ = Volume standard solution which has been used for amount of sample [ml]
$V_0$ = Volume standard solution, which has been used for blank test [ml]
$c_{eq;soll}$ = equivalent concentration standard solution
t = Titer standard solution
1,4007 = Factor for recalculating N content [%]
m = Weight Sample [g]

## 6.2 Protein calculation in %

With the following equation, the protein percentage of the sample can be calculated by means of a protein factor:

$$\omega_{\%,P} = \omega_{\%,N} \times 6{,}25$$

For different types of grains, there are different protein factors to be applied:

| Sample type | Protein factor |
|---|---|
| Barley | 6.25 |
| Oat | 5.7 or 6.25 |
| Pulses | 6.25 |
| Corn | 6.25 |
| Rye | 5.7 |
| Wheat | 5.7, 6.25 |
| | |

### 6.2.1 Repeatability

The maximum deviation of duplicate determinations respectively repeat determinations must not exceed the following values:

According to EU Regulation No. 152/2009 and GAFTA 4:1:
- 0.2 % absolute for contents less than 20 % raw protein
- 1.0 % relative to the higher result of contents between 20 % and 40 % raw protein
- 0.4 % absolute for contents higher than 40 % raw protein

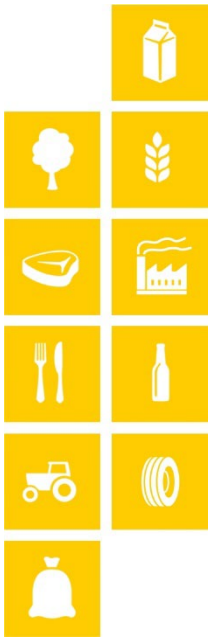
According to DIN EN ISO 20483:2014 the allowed difference between two individual results according to the same procedure on identical test material in the same laboratory with the same user applies, that the same device may not exceed the repeat limit r in more than 5% of the cases with the following equation:

$r = (0.0063 \times w_p) \times 2.8$ with $w_p$ = raw protein

## 7 Product table

According to „*Souci, Fachmann, Kraut: „Die Zusammensetzung der Lebensmittel“, medpharm Scientific Publishers, Stuttgart **1994**, CRC PRESS: Nährwert-Tabellen; 5. überarbeitete und ergänzte Auflage*“, the following sample amounts per sample types are recommended:

| Sample type | Sample amount [g] +/- 10 % | theor. content [%] Protein |
|---|---|---|
| Amaranth, Seeds | 1.5000 | 15.58 – 15.93 |
| Buckwheat, peeled | 1.5000 | 8.12 – 10.03 |
| Buckwheat groats | 1.5000 | 4.56 – 9.31 |
| Buckwheat whole grain flour | 1.2000 | 10.21 – 10.85 |
| Barley, no peel, whole grain | 1.5000 | 9.70 – 11.30 |
| Barley, peeled | 1.5000 | 7.80 – 12.00 |
| Barley groats | 1.5000 | 6.70 – 10.50 |
| Spelt flour, whole grain flour | 1.2000 | 14.36 |
| Spelt | 1.5000 | 11.59 |
| Spelt flour | 1.2000 | 8.90 – 11.90 |
| Oats, no peel, whole grain | 1.5000 | 11.90 – 13.20 |
| Oat meal | 1.5000 | 12.00 – 14.40 |
| Oat groats | 1.5000 | 11.40 – 16.30 |
| Millet, peeled | 1.5000 | 10.00 – 11.40 |
| Corn, whole grain | 1.5000 | 7.72 – 10.60 |
| Corn flakes | 1.2000 | 6.90 – 8.10 |
| Corn flour | 1.5000 | 6.72 – 10.00 |
| Quinoa | 1.2000 | 13.11 – 14.88 |
| Rice, polished | 1.5000 | 7.04 – 7.88 |
| Rice, polished, boiled, drained | 2.0000 | 2.10 |
| Rice flour | 1.2000 | 6.70 – 7.50 |
| Rye, whole grain | 1.5000 | 8.08 –11.10 |
| Rye flour | 1.2000 | 6.90 – 7.94 |
| Rye germs | 1.0000 | 39.30 – 44.70 |
| Sorghum | 1.5000 | 7.30 – 18.90 |
| Triticale | 1.5000 | 13.13 – 14.35 |
| Wheat, whole grain | 1.5000 | 10.02 – 12.98 |
| Seminola | 1.5000 | 8.43 – 10.03 |
| Wheat flour | 1.2000 | 9.00 – 12.00 |
| Wheat germs | 1.0000 | 26.14 |



## COMPREHENSIVE APPLICATION DATA BASE

C. Gerhardt offers a wide range of application notes for many methods and procedures. Please contact our application lab team via application@gerhardt.de for deeper information on:

- Nitrogen in food and feed samples according to Kjeldahl and Dumas
- Crude fibre, ADF and NDF in feed
- Fat in food and feed
- Alcohol determination
- Total cyanide in water
- Trace metal in soil and sludge
- COD determination in water
- Total nitrogen determination in water, soil and plants
- Many more application notes on request.

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