Kjeldahl Application

A.1.1.20. Soluble Proteins in KOH Solution – Soybean Flour

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1 Principle

Potassium hydroxide solubility tests are used to evaluate the quality of protein in soybean meals. The sample is dispersed in a solution of potassium hydroxide of approximately 12,5 pH, stirred and centrifuged. Then, the nitrogen content of the clarified liquid is determined according to the Kjeldahl method and compared with the value of crude protein of the original sample.

The analysis according to the Kjeldahl method is performed as follows: The organic bound nitrogen of the sample is digested in concentrated sulphuric acid in the presence of a salt to increase the boiling point which converts it 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 using an acid solution with a known concentration. The nitrogen content is calculated using the consumption of the titrant.

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

- ISO 14244:2014, Oilseed meals – Determination of soluble proteins in potassium hydroxide solution.

3 Chemicals und material

Quality grade p. a.

3.1. Water: demineralized or distilled
3.2. Paper weighing boats or weighing paper WP250 (Order number 1004939)
3.3. Catalyst tablets KJELCAT Cu 5,0 g K_2SO_4 + 0,5 g $CuSO_4$ x 5 H_2O (Order number 12-0328) or comparable
3.4. Concentrated sulphuric acid H_2SO_4
3.5. Caustic soda NaOH 32 %
3.6. Boric acid H_3BO_3 2 %
3.7. Indicator solution M5 (Merck) or comparable
3.8. Standard solution: hydrochloric acid c(HCl)= 0,1 mol/l (or sulphuric acid c(H2SO4) = 0,05 mol/l) or hydrochloric acid c(HCl)= 0,01 mol/l (or sulphuric acid c(H2SO4) = 0,005 mol/l)
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. Acetanilide
3.12. Potassium hydroxide solution c(KOH) = 0,036 mol/l

4 Instruments

- Rotor mill
- Analytical balance (reading accuracy at least 0.1 mg, preferably 0.01 mg)
- Magnetic stirrer and stir bars
- Beakers
- Pipettes
- Centrifuge or filter paper, nitrogen free
- Kjeldahl digestion system (KJELDATHERM, TURBOTHERM or flask heater for Kjeldahl flasks with enlarged neck)
- Fume Scrubber (TURBOSOG, VACUSOG or water jet pump)

- VAPODEST steam distillation system
  VAPODEST 200 to 450 are without titrator. The 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, with an indicator solution or with a pH electrode.
  The titration is performed automatically in case of VAPODEST 450 with external titrator or VAPODEST 500/500C with integrated titrator.

5 Procedure

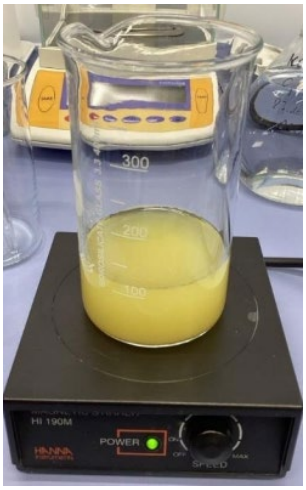
5.1 Sample Preparation

The soy flour is grinded through a 250 µm sieve.

The particle size of the sample affects the final result.

If the fat content is higher than 5%, it shall be defatted by cold extraction.

1,5 g of the soy flour is weighted and placed in a beaker. 75 ml of potassium hydroxide solution (3.12) are added, and the sample is stirred at minimum speed for 20 minutes to maintain all the solids in suspension.



Stirring of the solution for 20 minutes

The totality of the liquid is transferred to a centrifugal tube and centrifugated for 10 min at a relative acceleration of 800 g or is filtered.

No particle should be in suspension in the filtrate.

Just before the weighing, the sample is shaken thoroughly again. 25 ml aliquots of filtrate are added in the digestion tube. Each aliquot corresponds then to 0,5 g of the original milled sample.

Note: The determination of the nitrogen content according to Kjeldahl is performed in parallel with the same sample as described in the Application A.1.1.5.

5.2 Addition of the chemicals

The following chemicals are added in the digestion tube:

Chemicals	Amount per sample
KJELCAT (3.3)	2
Sulphuric acid (3.4)	20 ml

5.3 Digestion

Due to foaming that can occur during the digestion of liquid samples, the use of BS-400 digestion tubes (At. 12-0308) is recommended.

5.3.1 Digestion with KJELDATHERM

For digestions with a KJELDATHERM system, we recommend the following program parameters:

Phase	Step	hh:mm	Temp. [°C]	Power [%]	Lift	Suction	Fan	Cooling 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 the digestion manually and leave the samples for cooling.

If the sample is foaming too much, please choose the method "Water TKN".

5.3.2 Digestions with TURBOTHERM TTs

For digestions with a TURBOTHERM system, we recommend the following program parameters:

Note	Step	hh:mm	Power [%]	Suction
Heat-up of the system until boiling of the digestion solution	1/3	00:15	100	☑
Digestion	2/3	01:15	75	☑
Cooling	3/3	00:30	0	☑
Done		-	-	☐

If the sample is foaming too much, please choose the method "Foaming samples".

5.3.3 Suction of the digestion fumes

During the digestion, a fume scrubber (TURBOSOG or VACUSOG) must be activated. For the washing bottle we recommend filling approx. 1,2 L 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.

The fume scrubber should be left activated for about 30 minutes after having taken out the insert rack or after having turned off the heating.

Note: You can shorten the cooling down time of your samples by half with the KJELDATHERM Eco Kit (Order number 12-0778).

5.4 Distillation with VAPODEST

Once the samples are cooled down, a steam distillation is performed with the following program:

		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

5.5 Titration

Add 3-4 drops of the indicator solution M5 (3.7) to the receiver solution and titrate with the standard solution (3.8) until the colour changes from green to violet. If you determine the endpoint with a pH electrode, you do not have to add the indicator solution M5.

5.6 Blank value

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

5.7 Performance check

To check the analytical performance of your water steam distillation system, perform a distillation and titration of 0,12 g ammonium sulphate (3.10). The percentage of the recovered nitrogen must be higher than 99 % taking into account the titre of the standard solution. A recovery rate up to 101 % is still acceptable in sporadic cases.

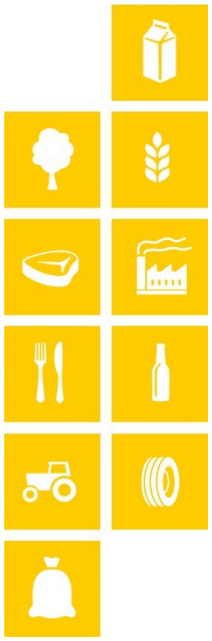
To verify the entire analysis process, perform the analysis with 0,1 g acetanilide (3.11) in the presence of 1 g sucrose (3.9). The recovery rate of nitrogen must be at least 99 %.

6 Calculation

$$KOH\ Protein\ Solubility\ [\%] = \frac{\frac{m_{N,filtrate\ analyzed}}{Portion\ of\ filtrate\ analyzed \times m_{orig.sample\ for\ treatment}} \times 100}{w_{N,orig.sample}} \times 100$$

With:

$m_{N.filtrate\ analyzed}$: nitrogen weight in filtrate analyzed [g]
Portion of filtrate analyzed: amount of filtrate analyzed/total amount of filtrate
$m_{orig.\ sample\ for\ treatment}$: weight of sample for KOH treatment [g]
$w_{N,orig.sample}$: nitrogen content in original sample [%]



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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