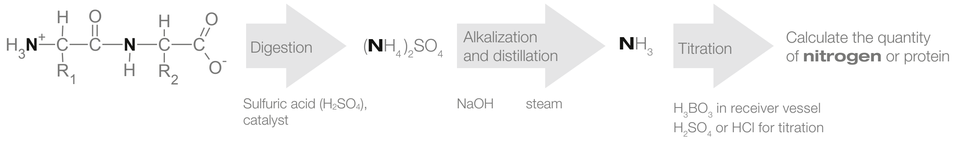
Kjeldahl determination of nitrogen and protein
Sample preparation, digestion, distillation and titration

What is determined?

Protein is determined by the analysis of the nitrogen content. From this, the protein content is calculated.
Protein consists of amino acids which contain nitrogen (N) in the amino group.



Protein contains: 15 – 18 % N
Average: 16 % N ⇨ factor 6.25 ⇨ 16 % nitrogen × 6.25 = 100 % protein

Other applications: TKN, ammonium, TVBN, urea, nicotine

Sample preparation and weighing

Milling
The samples have to be homogeneous.

Sample tube size

Micro: Reduction of chemicals by 80 %
300 mL: Standard Kjeldahl
500 mL: For high sample amounts

Weighing
The actual weight of a sample depends on the nitrogen content as well as on the inhomogeneity of the sample.

N [mg] per glass	Sample: weight [g]					Titrant concentration [N]			
	5	2	1	0.5	0.125	0.01	0.05	0.1	0.5
	N [%]					Titrant consumption for sample [mL]			
0.5	0.01	0.03	0.05	0.10	0.40	3.6			
2.0	0.04	0.10	0.20	0.40	1.60	14.3	2.9		
2.5	0.05	0.13	0.25	0.50	2.00		3.6	1.8	
7.0	0.14	0.35	0.70	1.40	5.60		10.0	5.0	
10.0	0.20	0.50	1.00	2.00	8.00		14.3	7.1	1.4
50.0	1.00	2.50	5.00	10.00	40.00				7.1
100.0	2.00	5.00	10.00	20.00	80.00				14.3

Procedure:
- Select N % of sample
- Select titrant concentration
- Choose weight in order that the titrant consumption can be expected between 3 and 17 mL

The amount of H_2SO_4 for digestion is given by

1. Conversion of K_2SO_4 to $KHSO_4$ (K_2SO_4 is a component of Kjeldahl Tablets)

   approx. 2 – 3 mL

2. Consumption by organic matter

Organic matter	H_2SO_4/ g [mL]	Example: Salami	e.g. for 1.5 g weight (weight · organic matter)
Fat	9.7	27.3 %	$1.5 \cdot 9.7 \cdot \frac{27.3}{100} = 3.97$ mL
Protein	4.9	20.6 %	$1.5 \cdot 4.9 \cdot \frac{20.6}{100} = 1.51$ mL
Carbohydrates	4.0	0.0 %	$1.5 \cdot 4.0 \cdot \frac{0.0}{100} = 0.0$ mL

3. Losses due to evaporation

   approx. 1 mL/h

4. Remaining volume

   = Quantity of the used Kjeldahl Tablets (e.g. 10 g Tablets = 10 mL H_2SO_4)

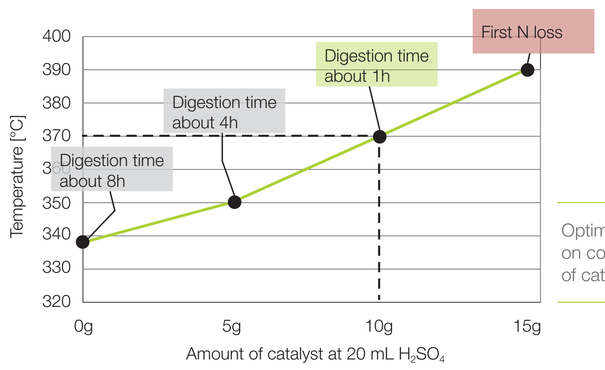
   H_2SO_4 volume = conversion + (total consumption by org. matter) + evaporation + remaining volume
   3 mL + (3.97 + 1.51 + 0.00) mL + 1 mL + 10 mL = 18.48 mL ~18 mL

Kjeldahl Tablets

Article	Order code	Composition	Weight
Titanium	11057980	3.5 g K_2SO_4 / 0.105 g $CuSO_4 \cdot 5\ H_2O$ 0.105 g TiO_2	3.71 g
Missouri	11057982	4.98 g K_2SO_4 / 0.02 g $CuSO_4 \cdot 5\ H_2O$	5 g
ECO	11057983	3.998 g K_2SO_4 / 0.002 g $CuSO_4$	4 g
Antifoam	11057984	0.97 g Na_2SO_4 / 0.03 g Silicon Antifoam	1 g
Titanium Micro	11057981	1.5 g K_2SO_4 / 0.045 g $CuSO_4 \cdot 5\ H_2O$, 0.045 g TiO_2	1.59 g
Copper Micro	11057985	1.5 g K_2SO_4 / 0.15 $CuSO_4 \cdot 5\ H_2O$	1.65 g

The aim of Kjeldahl Tablets is to accelerate the digestion by:
- Catalysis by metal salts
- Increasing the boiling point of H_2SO_4 by sulfate salts (K_2SO_4)

Attention: With boiling temperatures above 390 °C nitrogen losses are possible ⇨ leads to too low results!

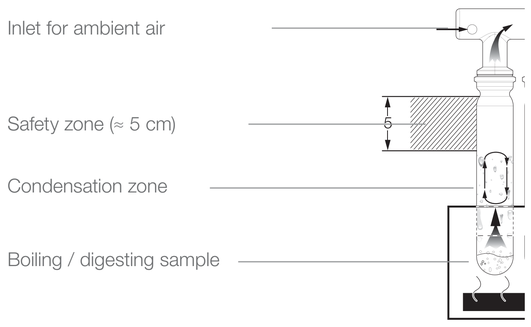


Optimal boiling point is 350 – 370 °C. Ideal digestion conditions are achieved with 2 mL H_2SO_4 to 1 g of catalyst.

The digestion

Digestion: $(C_nH_nN_nO_n) + H_2SO_4 \rightarrow CO_2 + SO_2 + H_2O + NH_4^+$

Organic matter is destroyed by boiling in concentrated sulfuric acid. Kjeldahl Tablets raise the boiling point and accelerate the process.



Optimal conditions are achieved when the condensation zone remains 5 cm below the constriction of the sample tube.

The distillation and boric acid titration

Distillation: $NH_4^+ + OH \rightleftharpoons NH_3\ (Gas) + H_2O$

The digestion mixture is alkalized with NaOH prior to distillation to free up the ammonia. The ammonia is steam distilled into an acidic receiver solution.

Step	Why	How much	Rule-of-the-thumb
Step 1: Dilution			
H_2O dest.	Dilution of the strongly acidic solution, prevents violent reactions	25 – 90 mL	4 mL per mL used H_2SO_4
Step 2: Alkalinization			
NaOH 32 %	Conversion of NH_4^+ in NH_3 (gaseous)	15 – 90 mL	4.5 mL per mL used H_2SO_4
Step 3: Preparation of the receiver			
H_3BO_3 (pH 4.65)	To collect the distilled NH_3. NH_3 is bound as borate complex $NH_4B(OH)_4$.	40 – 70 mL	2 % H_3BO_3 with KCl for low N contents 0.02 – 6.75 mg/sample tube 4 % H_3BO_3 for medium and high N content 6.75 – 125 mg/sample tube
Step 4: Distillation			
Water steam (100 %)	Separation of NH_3 by boiling of the sample	180 – 300 s	Distillation time: 180 s with KjelMaster 240 s with others
Step 5: Collection			
NH_3	In boric acid receiver of pH 4.65		Condenser oulet tube and electrode must be completely immersed

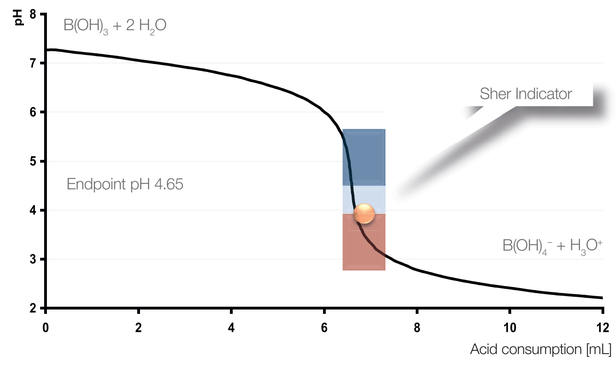
The titration

Receiver: $B(OH)_3 + NH_3 + H_2O \rightleftharpoons NH_4^+ + B(OH)_4^-$

HX = H_2SO_4 or HCl

Titration: $B(OH)_4^- + HX \rightarrow X^- + B(OH)_3 + H_2O$

The pH in the acidic receiver solution rises upon addition of ammonia. The nitrogen and protein content is then determined by titration of the borate complex.



Endpoint titration boric acid: $B(OH)_3 + 2\ H_2O \rightleftharpoons B(OH)_4^- + H_3O^+$ ($pK_a = 9.24$)

The result calculation

$$\%N = \frac{[V(1) - V(Bl)] \cdot F \cdot c \cdot f \cdot M(N)}{m \cdot 1000} \cdot 100\,\%$$

% P = % N · PF

V(1): consumption of titrant, sample [mL]
V(Bl): average consumption of titrant, blank [mL]
F: molar reaction factor (1 = HCl, 2 = H_2SO_4)
c: concentration of titrant [mol /L]
f: factor of titrant
M(N): molecular weight of N (14,007 [g/mol])
m: sample weight [g]
1000: conversion factor (mL in L)
PF: protein factor
% N: % of weight of N
% P: % of weight of protein

Example:
Titrant: 0.25 mol/L H_2SO_4
f = 1.000
Weighing: 0.750 g
Blank value: 1.0 mL
Titration volume for sample: 7.5 mL

$$\frac{(7.5\,\text{mL} - 1\,\text{mL}) \cdot 2 \cdot 0.25 \cdot 1 \cdot 14.007 \cdot 100\,\%}{0.750\,\text{g} \cdot 1000} = 6.07\,\%\ \text{N}$$

6.07 % N · 6.25 = 37.94 % P

Factors for the conversion of nitrogen in protein

Food	Amount N(%)	protein factor	Food	Amount N(%)	protein factor
Egg / meat / fish	16.0	6.25	Almonds	19.3	5.18
Milk / ice cream / casein	15.7	6.38	Peanuts / brasil nuts	18.3	5.46
Wheat / baked prod. / oat	17.5	5.70	Tree nut / coconut	18.9	5.30
Rye / soy	17.5	5.70	Rice	19.0	5.26
Barley / maize / pulses	16.0	6.25	Beer / brewing sugars	16.0	6.25

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