Application Note No. 110/2013
Nitrogen & protein determination in corn, flour and soy

KjelDigester K-449, KjelMaster K-375 with KjelSampler K-376:
Nitrogen and Protein Determination in Corn, Flour and Soy according to the Kjeldahl Method

1 Introduction

An easy and reliable method for the determination of total nitrogen and protein in corn, flour and soy, according to ISO 20483:2006 and LFGB §64 L15.00-3, is introduced below. The samples are digested using the KjelDigester K-449. The distillation and boric acid titration are performed with the KjelMaster K-375 with KjelSampler K-376. The combination of the new KjelDigester and the KjelMaster system K-375/K-376 increases the sample throughput.

2 Equipment

- KjelDigester K-449 (the parameters used are also valid for K-446)
- Scrubber K-415 TripleScrub ^{ECO}
- KjelMaster K-375 with KjelSampler K-376
- Mixer, Retsch Grindomix GM200
- Analytical balance (accuracy ± 0.1 mg)

3 Chemicals and Materials

Chemicals:

- Sulfuric acid conc 98 %, Merck (1007482500)
- Titanium, BUCHI Kjeldahl Tablet (11057980)
- Sodium hydroxide 32 %, Brenntag (81980-452)
- Boric acid 4 %, 400 g boric acid, Brenntag (80948-155) diluted to 10 L with deionized water, pH adjusted to 4.65
- Sulfuric acid 0.1 mol/L, Fluka (35357)
- Neutralization solution for the Scrubber: 600 g sodium carbonate, calcined, technical, Synopharm (0179420) about 2 mL ethanol and a spatula tip of bromthymol blue, Fluka (18460) diluted to 3 L with distilled water
- D/L tryptophan, assay 99 %, Fluka (162698)

For a safe handling please pay attention to all corresponding MSDS!

Samples:

- Corn with a protein content of 9 g/100 g [1]
- Wheat flour with a labelled protein content of 14 g/100 g
- Soy beans with a protein content of 35 g/100 g [1]

The samples were purchased at a local supermarket.

4 Procedure

The determination of nitrogen and protein in corn, wheat flour and soy includes the following steps:

- Drying of the reference substance tryptophan [2, 3]
- Homogenization of the sample by grinding
- Digestion of the sample, using K-449 (K-446 respectively)
- Distillation and titration of the sample, using KjelMaster system K-375/K-376

4.1 Digestion method – tryptophan (verification of the method)

1. Start the KjelDigester K-449 according to the parameters listed in Table 2
2. Place 0.15 g tryptophan in a 300 mL sample tube
3. Add 2 Titanium Tablets and 15 mL of sulfuric acid (conc. 98 %)
4. Prepare additional blanks, chemicals without sample
5. Connect the Scrubber K-415 to the K-449 for absorbing the acid fumes created during digestion
6. Insert the rack with the samples into the cooling position and mount the suction module onto the samples, immediately start the digestion according to the parameters listed in Table 2.
7. Let the samples cool down when the digestion is completed.

4.2 Digestion method – samples

1. Start the KjelDigester K-449 according to the parameters listed in Table 2
2. Place each sample in a 300 mL sample tube as described in Table 1

Table 1: Weight for each sample

Sample	Weight [g]
Corn	1.0
Wheat flour	1.0
Soy beans	0.5

3. Add 2 Titanium Tablets and 15 mL of sulfuric acid (conc. 98%) to each tube
4. Prepare additional blanks, chemicals without sample
5. Connect the Scrubber K-415 to the K-449 for absorbing acid fumes created during digestion
6. Insert the rack with the samples into the cooling position and mount the suction module onto the samples, immediately start the digestion according to the parameters listed in Table 2.

Table 2: Temperature profile for digestion with the K-449

Step	Temperature [°C]	Time [min]
1	300	0
2	420	125
Cooling	–	35

NOTE: If the liquid inside the sample tube is not clear and blue-green, digest for additional 15 min at 420° C.

7. Let the samples cool down when the digestion is completed.

4.3 Distillation and titration

Distill the samples according to the parameters listed in Table 3.

Table 3: Distillation and titration with the KjelMaster system K-375/K-376

Method parameters KjelMaster K-375

H_2O volume	50 mL	Titration solution	H_2SO_4 0.1 mol/L
NaOH volume	60 mL	Sensor type	Potentiometric
Reaction time	5 s	Titration mode	Online
Distillation mode	Fixed time	Titration start time	120 s
Distillation time	180 s	Measuring mode	Endpoint pH
Stirrer speed distillation	5	Endpoint pH	4.65
Steam output	100 %	Stirrer speed titration	7
Titration type	Boric acid	Titration start volume	0 mL
Receiving solution vol.	50 mL	Titration algorithm	Optimal

NOTE: The sample throughput for this application was increased by using the Titration mode “Online”.

4.4 Calculation

The results are calculated as a percentage of nitrogen. In order to calculate the protein content of the sample, the nitrogen content is multiplied with a sample-specific protein factor. The following equations (1), (2), and (3) are used to calculate the results. For the reference substance, the purity of the tryptophan is considered in equation (4).

$$w_N = \frac{(V_{Sample} - V_{Blank}) \cdot z \cdot c \cdot f \cdot M_N}{m_{Sample} \cdot 1000} \quad (1)$$

$$\%N = w_N \cdot 100\ \% \quad (2)$$

$$\%P = w_N \cdot PF \cdot 100\ \% \quad (3)$$

$$\%N_{Try} = \frac{\%N \bullet 100}{P} \quad (4)$$

w_N : weight fraction of nitrogen

V_{Sample} : amount of titrant for the sample [mL]

V_{Blank} : mean amount of titrant for the blank [mL]

z : molar valence factor (1 for HCl, 2 for H_2SO_4)

c : titrant concentration [mol/L]

f : titrant factor (for commercial solutions normally 1.000)

M_N : molecular weight of nitrogen (14.007 g/mol)

m_{Sample} : sample weight [g]

1000 : conversion factor [mL/L]

%N : percentage of weight of nitrogen

$\%N_{Try}$: percentage of weight of nitrogen corrected for the purity of reference substance tryptophan [%]

%P : percentage of weight of protein

P : purity of the reference substance tryptophan [%]

PF : sample-specific protein factor (6.25 for corn, wheat flour and 5.71 for soy beans)

5 Results

5.1. Recovery of tryptophan

The results of nitrogen determination and recovery for tryptophan analysis (assay > 99 %) are presented in Table 4. The nominal value of tryptophan is 13.72 % nitrogen. The recoveries are within the specification of ≥ 99 %. [2, 3]

Table 4: Results of the recovery of nitrogen in tryptophan

Tryptophan	m_{Sample} [g]	V_{Sample} [mL]	$\%N_{Try}$	Recovery [%]
Sample 1	0.1530	7.566	13.69	99.9
Sample 2	0.1511	7.466	13.67	99.8
Sample 3	0.1489	7.356	13.66	99.8
Sample 4	0.1446	7.122	13.61	99.4
Average [%]	–	–	13.66	99.7
Rsd [%]	–	–	0.2	0.2

The mean blank volume (V_{Blank}) was 0.166 mL (n = 4).

5.2 Protein determination in corn, wheat flour and soy beans

The results of the determination of nitrogen and protein contents are presented in Tables 5 – 7.

Table 5: Results of the determination of nitrogen and protein in corn (protein content 9 g/100 g [1])

Corn	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	1.1415	6.188	1.478	9.24
Sample 2	1.1273	6.200	1.499	9.37
Sample 3	0.9598	5.328	1.509	9.42
Sample 4	0.9659	5.316	1.494	9.33
Average [%]	–	–	1.494	9.34
Rsd [%]	–	–	0.8	0.8

The mean blank volume (V_{Blank}) was 0.166 mL (n = 4).

Table 6: Results of the determination of nitrogen and protein in wheat flour (labelled protein content 14 g/100 g)

Wheat flour	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.9946	8.404	2.320	14.50
Sample 2	1.0662	8.995	2.320	14.50
Sample 3	1.0098	8.524	2.319	14.49
Sample 4	0.9434	7.974	2.319	14.49
Average [%]	–	–	2.320	14.50
Rsd [%]	–	–	0.0	0.0

The mean blank volume (V_{Blank}) was 0.166 mL (n = 4).

Table 7: Results of the determination of nitrogen and protein in soy beans (protein content 35 g/100 g [1])

Soy beans	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.5023	11.539	6.343	36.22
Sample 2	0.5404	12.484	6.385	36.46
Sample 3	0.5282	12.083	6.320	36.09
Sample 4	0.5338	12.225	6.329	36.14
Average [%]	–	–	6.344	36.23
Rsd [%]	–	–	0.5	0.5

The mean blank volume (V_{Blank}) was 0.166 mL (n = 4).

6 Comparison to Standard Methods

This application note is based on the standard method ISO 20483:2006 and LFGB §64 L15.00-3 with minor differences. These differences are shown in Table 8.

Table 8: Differences to the official regulations

	Application note	Regulations	Notes / Impact
Sulfuric acid	15 mL	20 mL	No impact, same ration of sulfuric acid/catalyst.
Titration solution	H_2SO_4 0.2N	H_2SO_4 0.1N	No impact consumption of the titration solution should be between 3-17 mL.

7 Conclusion

The determination of nitrogen and protein in corn, wheat flour and soy beans using the KjelDigester K-449 and KjelMaster system K-375/K-376 provides reliable and reproducible results. These results correspond well to the labelled values with low relative standard deviations (rsd). The recovery with tryptophan was 99.7 % (rsd = 0.2 %), which was within the specification of ≥ 99 % [2, 3].
With the KjelDigester K-449 the digestion process (including preheating, digestion and cooling) is very fast and is fully automated. Together with the fully-automatic KjelMaster system K-375/K-376, the time to result is significantly reduced and it offers fully walk-away convenience.

8 References

[1] Souci Fachmann Kraut: *Die Zusammensetzung der Lebensmittel. Nährwert-Tabellen*, CRS Press, 7., revidierte und ergänzte Auflage. 2008
[2] ISO 20483:2006 Cereals and pulses – Determination of the nitrogen content and calculation of the crude protein content – Kjeldahl method
[3] LFGB §64 L15.00-3

Kjeldahl Calculator App

Operation Manual of KjelDigester K-446/K-449
Operation Manual of Scrubber K-415
Operation Manual of KjelMaster system K-375/K376