Application Note No. 107/2013
Nitrogen & protein determination in beer and malt

KjelDigester K-449, KjelMaster K-375 with KjelSampler K-376:
Accelerated Nitrogen and Protein Determination in Beer and Malt with Kjeldahl Tablets and Hydrogen Peroxide

1 Introduction

An easy and reliable method for the determination of total nitrogen and protein in beer and malt by the use of hydrogen peroxide, 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 accelerated digestion method, using the Kjeldahl Tablet Titanium in combination with hydrogen peroxide, 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)
- Volumetric pipette, 10 mL

Safety accessories:

- User protection shield, BUCHI (11057889)
- Hirschmann bottle top dispenser ceramus® 5-30 mL, VWR (613-3243) with ceramus® discharge tube, spiral-shaped, VWR (612-0917)

Figure 1: Safety accessories for the digestion with hydrogen peroxide and Kjeldahl Tablet Titanium

3 Chemicals and Materials

Chemicals:

- Sulfuric acid conc 98 %, Merck (1007482500)
- Titanium, BUCHI Kjeldahl Tablet (11057980)
- Hydrogen peroxide, 30 %, Fluka (95302)
- 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 0.05 mol/L, Fluka (35358)
- 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
- Acetanilide, assay 99 %, Fluka (112933)

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

Samples:

- Lager beer I with a labelled protein content of < 0.5 g/100 mL
- Lager beer II with a labelled protein content of 0.5 g/100 mL
- Malt I with a protein content of 12.5 g/100 g
- Malt II with a protein content of 9.0 g/100 g

The beer samples were purchased at a local supermarket.

4 Procedure

The determination of nitrogen and protein in beer and malt includes the following steps:

- Homogenization of the sample by shaking or grinding, depending on the matrix
- Removing of the CO_2 of the beer samples by shaking or stirring
- 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 – acetanilide (verification of the method)

1. Start the KjelDigester K-449 according to the parameters listed in Table 2
2. Place 0.1 g acetanilide in a 300 mL sample tube
3. Add 1 Titanium tablet and 10 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. Place the rack under the fume hood and affix the protection shield
7. *Slowly* add 6 mL hydrogen peroxide (30 %) with the dispenser down the glass wall of the sample tube, wait until the fuming stops and the reaction subsides.
8. Insert the rack containing the user protection shield and the samples into the cooling position and mount the suction module onto the samples, immediatiely start the digestion according to the parameters listed in Table 2.
9. 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	Amount
Beer	10 mL
Malt	0.4 g

3. Add 1 Titanium tablet and 10 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. Place the rack under the fume hood and affix the protection shield
7. *Slowly* add 6 mL hydrogen peroxide (30 %) with the dispenser down the glass wall of the sample tube, wait until the fuming stops and the reaction subsides.
8. Insert the rack containing the user protection shield and the samples into the cooling position and mount the suction module onto the samples, immediatiely 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	360	0
2	420	65
Cooling	–	25

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

NOTE: The samples should be clear-green immediately after the digestion. A darkening of the clear liquid samples during the cooling down process is possible but does not affect the results.

9. 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 0.05 mol/L
NaOH volume	45 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.	60 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 acetanilide is considered in equation (4).

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

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

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

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

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

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

%P : percentage of weight of protein

P : purity of the reference substance acetanilide [%]

PF : sample-specific protein factor (6.25 for beer and malt)

5 Results

5.1. Recovery of acetanilide

The results of nitrogen determination and recovery for acetanilide analysis (assay > 99 %) are presented in Table 4. The nominal value of acetanilide is 10.36 % nitrogen. The recoveries are within the specification of 98 – 102 % [1].

Table 4: Results of the recovery of nitrogen in acetanilide

Acetanilide	m_{Sample} [g]	V_{Sample} [mL]	$\%N_{Ac}$	Recovery [%]
Sample 1	0.1009	7.648	10.38	100.2
Sample 2	0.1006	7.655	10.43	100.6
Sample 3	0.1091	8.264	10.40	100.4
Sample 4	0.1050	7.998	10.45	100.8
Average [%]	–	–	10.42	100.5
Rsd [%]	–	–	0.3	0.3

The mean blank volume (V_{Blank}) was 0.243 mL (n = 4).

5.2 Protein determination in beer and malt

The results of the determination of nitrogen and protein contents in beer and malt are presented in Tables 5 – 8.

Table 5: Results of the determination of nitrogen and protein in beer (declared protein content <0.5 g/100 mL)

Beer I	m_{Sample} [mL]	V_{Sample} [mL]	%N	P [g/100 mL]
Sample 1	10	4.899	0.0652	0.408
Sample 2	10	4.891	0.0651	0.407
Sample 3	10	4.903	0.0653	0.408
Average [%]	–	–	0.0652	0.408
Rsd [%]	–	–	0.1	0.1

The mean blank volume (V_{Blank}) was 0.243 mL (n = 4).

Table 6: Results of the determination of nitrogen and protein in beer (declared protein content 0.5 g/100 mL [2])

Beer II	m_{Sample} [mL]	V_{Sample} [mL]	%N	P [g/100mL]
Sample 1	10	4.556	0.0604	0.378
Sample 2	10	4.590	0.0609	0.381
Sample 3	10	4.592	0.0609	0.381
Average [%]	–	–	0.0607	0.380
Rsd [%]	–	–	0.5	0.5

The mean blank volume (V_{Blank}) was 0.243 mL (n = 4).

Table 7: Results of the determination of nitrogen and protein in malt (declared protein content 12.5 g/100 g)

Malt I	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.4645	6.925	2.015	12.6
Sample 2	0.4079	6.107	2.014	12.6
Sample 3	0.3984	5.927	1.999	12.5
Average [%]	–	–	2.009	12.6
Rsd [%]	–	–	0.5	0.5

The mean blank volume (V_{Blank}) was 0.243 mL (n = 4).

Table 8: Results of the determination of nitrogen and protein in malt (protein content 9.0 g/100 g)

Malt II	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.4226	4.655	1.463	9.14
Sample 2	0.4245	4.655	1.456	9.10
Sample 3	0.3969	4.417	1.473	9.20
Average [%]	–	–	1.464	9.15
Rsd [%]	–	–	0.6	0.6

The mean blank volume (V_{Blank}) was 0.243 mL (n = 4).

6 Conclusion

The determination of nitrogen and protein in beer and malt 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) and a short digestion time of 65 min. The recovery with acetanilide was 100.5 % (rsd = 0.3 %), which was within the specification of 98 - 102 % [1].
In combination with the accelerated digestion method using the KjelDigester K-449, Kjeldahl Tablet Titanium and hydrogen peroxide, the time needed for sample analysis is significantly reduced and therefore throughput increased.
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.

7 References

[1] Application Note 100/2013, Nitrogen Determination in Reference Substances – Operational Qualitiy Check Procedure
[2] Souci Fachmann Kraut: *Die Zusammensetzung der Lebensmittel. Nährwert-Tabellen*, CRS Press, 7., revidierte und ergänzte Auflage. 2008

KjelCalc App

Application Note 108/2013, Nitrogen and Protein Determination in Beer and Malt according to Kjeldahl Method

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