Application Note
No. 199/2015

Nitrogen and protein determination in meat products

KjelDigester K-449, KjelMaster K-375 with KjelSampler K-376:
Nitrogen and Protein Determination in Meat Products according to the Kjeldahl Method.

1 Introduction

A reliable method for the determination of nitrogen and protein in meat products, i.e. Salami, smoked ham and boiled beef sausage according to AOAC 981.10, is introduced below [1]. The samples are digested using the KjelDigester K-449. The distillation and boric acid titration are performed using the KjelMaster system K-375 / K-376. Equivalent to the international standard [1], the measuring method of the boric acid titration is colorimetric. Therefore, a mixed indicator accoding to Sher is added to the boric acid solution and the KjelMaster K-375 is equipped with the colorimetric sensor. The combination of the KjelDigester, the KjelMaster system K-375 / K-376 and the novel “online” titration mode increases the sample throughput to up to 120 samples per workday (9 h).

2 Equipment

- KjelDigester K-449 (the parameters used are also valid for the K-446)
- User protection shield (BUCHI 11057889)
- Scrubber K-415 TripleScrub ECO with TKN Set (BUCHI 11057333)
- KjelMaster K-375 with colorimetric sensor
- KjelSampler K-376 (the parameters used are also valid for the K-377)
- Analytical balance (accuracy ± 0.1 mg)
- Digestion rods, boiling aid (BUCHI 043087)
- Volumetric pipettes

3 Chemicals and Materials

- Sulfuric acid conc 98 %, analytical reagent, Beijing Chemical Works
- Titanium, BUCHI Kjeldahl Tablet (11057980)
- Hydrogen peroxide, 30 %, analytical reagent, Beijing Chemical Works
- Sodium hydroxide 32 %, analytical reagent, Sinoharm chemical reagent
- Boric acid 4 %, 200 g boric acid (analytical reagent, Tianjin guangfu fine chemical research institute) diluted to 5 L with deionized water, dissolve completely, add 12.5 mL of mixed Sher indicator, BUCHI (003512), adjust pH to 4.65
- Sulfuric acid 0.5 mol/L, Aladdin (S112267-1L)
- Neutralization solution for the Scrubber K-415: 600 g sodium carbonate (analytical reagent, Sinoharm chemical reagent) about 2 mL ethanol and a spatula tip of bromthymol blue (analytical reagent, Tianjin Kemiou chemical reagent) diluted to 3 L with distilled water
- Glycine, assay 99.5 %, Sinoharm chemical reagent

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

Samples:
- Salami with a labelled protein content of 25.2 g/100 g
- Smoked ham with a labelled protein content of 16.0 g/100 g
- Boiled beef sausage with a labelled protein content of 15.8 g/100 g

The samples were purchased at at a local supermarket.

4 Procedure

The determination of nitrogen and protein in meat products includes the following steps:

- Homogenizsation of the samples by the Mixer B-400
- 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 Homogenization of the sample, using the Mixer B-400

1. Take approx. 50 g of deep-freezed sample
2. Cut the meat into small pieces, sample size approx. 1.5 cm × 1.5cm
3. Place the sample into the glass vessel of the Mixer B-400 (not higher than the BUCHI logo)
4. Homogenize the sample for 5 s
5. If some big fragments remaine, homogenize again for 5 s
6. Wait until the sample is warmed to room temperature

4.2 Digestion method – glycine (verification of the method)

1. Start the KjelDigester K-449 according to the parameters listed in Table 2
2. Place 0.1 g glycine in a 300 mL sample tube
3. Add two 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. Place the rack under the fume hood and attch the protection shield
7. Dropwise add 8 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 protection shield and 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.
9. Let the samples cool down when the digestion is completed

4.3 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, the corresponding weight is described in Table 1

Table 1: Weight for each sample.

Sample	Weight [g]
Salami	0.5
Smoked ham	1
Boiled beef sausage	1

3. Add two 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. Place the rack under the fume hood and affix the user protection shield
7. Dropwise add 8 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, 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	320	10
2	420	55
Cooling	–	35

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.

4.4 Distillation and titration

For colorimetric titration it is necessary to determine the setpoint of the boric acid solution in advance to the blank and sample determinations. To adjust the device to the current conditions it is necessary to determine the setpoint every day before starting sample determinations, and when the method is changed or fresh chemicals are used.
The detailed procedure, including the preparation of the sensor, is described in the Technical Note 179/2015 “Colorimetric titration procedure using Sher indicator” [2].
The setpoint was measured three times.

- 1st setpoint → preheating
- 2nd setpoint → 1st measurement
- 3rd setpoint → 2nd measurement, confirms the 1st measurement

The last setpoint measurement is used as endpoint for all following determinations including priming, blanks and samples.

1. Determine the setpoint and check it’s range and deviation:
   Select all parameters for the setpoint determination according to Table 3.

Table 3: Parameters for setpoint determination.

Parameter	Setting
Preheating before setpoint	Yes
Setpoint runs	3
Setpoint cycle	Via sampler
Boric acid	4 %
Indicator	Sher
Method	Select the same method as for sample determination

NOTE: The selected method, boric acid and indicator for setpoint determination must be identical to the method used for sample determination.

2. Check the setpoint range and deviation
   - The determined setpoints should be in a range of 700-900 mV
   - The deviation between the two last measured setpoints should be ≤ 20 mV
3. Perform a priming to remove all residues
4. Determine blanks according to the parameters listed in Table 5

5. Determine samples according to the parameters listed in Table 5.

Table 4: Setpoint measurements and deviation.

Setpoint 2nd	808.2 mV
Setpoint 3rd	807.3 mV
Deviation	0.9 mV

Table 5: Distillation and titration with the KjelMaster system K-375 / K-376.

H2O volume	50 mL	Titration solution	H_2SO_4 0.1 mol/L
NaOH volume	60 mL	Sensor type	Colorimetric
Reaction time	5 s	Titration mode	Online
Distillation mode	Fixed time	Titration start time	120 s
Distillation time	180 s	Measuring mode	Setpoint
Stirrer speed distillation	5	Stirrer speed titration	10
Steam output	100 %	Titration start volume	0 mL
Titration type	Boric acid	Titration algorithm	Normal
Receiving solution vol.	60 mL		

NOTE: The sample throughput for this application was increased by using the “Online” titration mode: By applying the “Online” titration the time for the distillation and titration process is reduced to about 5 minutes per analysis because titration starts during the distillation is still in progress.

4.5 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 glycine is considered in equation (4).

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

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

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

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

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

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

%P : percentage of weight of protein

P : purity of the reference substance glycine [%]

PF : sample-specific protein factor (6.25 for meat products)

5 Results

5.1. Recovery of glycine

The results of nitrogen determination and recovery for glycine analysis (assay ≥ 99.5 %) are presented in Table 6. The nominal value of glycine is 18.66 % nitrogen. The recoveries are within the specification of 98 – 100 % [1].

Table 6: Results of the recovery of nitrogen in glycine.

Glycine	m_{Sample} [g]	V_{Sample} [mL]	$\%N_{Gly}$	Recovery [%]
Sample 1	0.0814	5.709	18.62	99.8
Sample 2	0.0793	5.564	18.60	99.7
Sample 3	0.0823	5.774	18.64	99.9
Sample 4	0.0831	5.776	18.46	98.9
Average [%]	–	–	18.58	99.6
Rsd [%]	–	–	0.4	0.4

The mean blank volume (V_{Blank}) was 0.299 mL (n = 3).

5.2 Protein determination in meat products

The results of the determination of nitrogen and protein contents in different meat products are presented in Tables 7-9.

Table 7: Results of the determination of nitrogen and protein in Salami (lablled protein content 25.2 g/100 g)

Salami	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.6656	10.730	4.40	27.5
Sample 2	0.6725	10.728	4.35	27.2
Sample 3	0.5616	8.948	4.32	27.0
Sample 4	0.6641	10.878	4.47	27.9
Average [%]	–	–	4.38	27.4
Rsd [%]	–	–	1.5	1.5

The mean blank volume (V_{Blank}) was 0.285 mL (n = 3).

Table 8: Results of the determination of nitrogen and protein in smoked ham (lablled protein content 16.0 g/100 g)

Smoked ham	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	1.1050	9.286	2.28	14.3
Sample 2	0.9530	8.027	2.28	14.2
Sample 3	1.0492	8.819	2.28	14.2
Sample 4	0.9086	7.720	2.29	14.3
Average [%]	–	–	2.28	14.3
Rsd [%]	–	–	0.3	0.3

The mean blank volume (V_{Blank}) was 0.285 mL (n = 3).

Table 9: Results of the determination of nitrogen and protein in boiled beef sausage (protein content 15.8 g/100 g)

Boiled beef sausage	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	1.1193	10.455	2.54	15.9
Sample 2	1.1242	10.518	2.55	15.9
Sample 3	0.9681	8.7970	2.46	15.4
Sample 4	0.9701	9.0350	2.52	15.8
Average [%]	–	–	2.52	15.7
Rsd [%]	–	–	1.6	1.6

The mean blank volume (V_{Blank}) was 0.299 mL (n = 3).

6 Comparison to AOAC 981.10

This application note is based on the standard method AOAC 981.10 with minor differences. These differences are shown in Table 10.

Table 10: Differences to AOAC 981.10.

	Application note	AOAC 981.10	Notes/Impact
Catalyst	2 × 3.7 g Tablets Composition 94.4 % K_2SO_4 2.8 % TiO_2 2.8 % $CuSO_4*5H_2O$	2 × 3.7 g Tablets Composition 95.2 % K_2SO_4 4.8 % HgO	No impact, no use of toxic mercury.
Hydrogen peroxide	8 mL (conc. 30 %)	3 mL (conc. 30 %)	Higher volume of hydrogen peroxide instead of mercury as catalyst to speed up the digestion.
NaOH	60 mL (conc. 32 %)	50 mL (conc. 40 %) containing thiosulfate	No impact, no use of thiosulfate solution necessary, because no use of mercury as catalyst.
Boric acid	60 mL	25 mL	In the conducted experiments a higher amount of boric acid was used then described in standard methods as the tip of the condenser outlet and the electrode have to be immersed in the solution.
Titration solution	H_2SO_4 0.1 mol/L	HCl 0.2 mol/L	No impact
Titration	colorimetric	colorimetric	No impact, the results are equal

7 Conclusion

The determination of TKN (Total Kjeldahl Nitrogen) in meat products using the KjelDigester K-449, the KjelMaster system K-375 / K-376 and the colorimetric sensor provides reliable and reproducible results. The found protein content of Salami, smoked ham and boild beef sausage correspond well to the labelled values of the meat products. The recovery with glycine, a standard to control the reliability of the measurments, was 99.6 % (rsd = 0.4 %), which was within the specification of 98 - 100 % [1].
With the KjelDigester K-449 the digestion process (including preheating, digestion and cooling) is very fast and is fully automated. Together with the automatic KjelMaster system K-375 / K-376 and the novel “Online” titration mode, the time to result is significantly reduced and it offers fully walk-away convenience.

8 References

[1] AOAC 981.10 Crude Protein in Meat
[2] Technical Note No.179/2015 Colorimetric titration procedure using Sher indicator