Application Note – N°. 1000/2025

Sample Application Note

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1. Introduction

An easy and reliable method for the determination of total nitrogen and protein in dairy products, according to ISO 8968-2 and AOAC 991.20, 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:

- Skimmed milk with a labelled protein content of 3.2 g/100 mL.
- Cream with a labelled protein content of 2.5 g/100 mL.
- Lassi drink with a labelled protein content of 2.5 g/100 mL.
- Parmesan cheese with a labelled protein content of 33 g/100 g.

The samples were purchased at a local supermarket.

4. Procedure

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

- Homogenization of the sample by shaking or grinding depending on the matrix.
- 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 0.12 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

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

Table 1: Weight for each sample.

Sample	Weight [g]
Skimmed milk	3.0
Cream	2.0
Lassi drink	2.5
Parmesan cheese	0.4

1. Add 2 Titanium Tablets and 15 mL of sulfuric acid (conc. 98%) to each tube.
2. Prepare additional blanks, chemicals without sample.
3. Connect the Scrubber K-415 to the K-449 for absorbing acid fumes created during digestion.
4. Insert the rack with the samples into the lift and 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	340	15
3	420	105
Cooling	-	35

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

Let the samples cool down and start the distillation according to Table 3.

4.3 Distillation and titration

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

Table 3: Parameters for 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	100 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

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).

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

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

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

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

$_wN$: 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).
MN: Molecular weight of nitrogen (14.007 g/mol).
m_{Sample}: Sample weight [g].
1000: Conversion factor [mL/L].
%N: Percentage of weight of nitrogen.
%NTry: 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 (6.38 for dairy products).

5. Result

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 ≥ 98%. [1,2,3]

Table 4: Results of the recovery of nitrogen in tryptophan.

Tryptophan	mSample [g]	V_{Sample} [mL]	%NTry	Recovery [%]
Sample 1	0.1420	7.026	13.66	99.6
Sample 2	0.1250	6.208	13.67	99.6
Sample 3	0.1256	6.267	13.73	100.1
Sample 4	0.1225	6.113	13.73	100.1
Average [%]	-	-	13.70	99.8
Rsd [%]	-	-	0.3	0.3

The mean blank volume (V_{Blank}) was 0.171 mL (n = 4).

5.2 Protein determination in dairy products

The results of the determination of nitrogen and protein contents in dairy products are presented in Tables 5-8.

Table 5: Results of the determination of nitrogen and protein in skimmed milk (declared protein content 3.2 g/100 mL).

Skimmed milk	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	2.9964	5.961	0.5413	3.45
Sample 2	3.0463	6.051	0.5407	3.45
Sample 3	3.0224	6.039	0.5439	3.47
Average [%]	-	-	0.5420	3.46
Rsd [%]	-	-	0.3	0.3

The mean blank volume (V_{Blank}) was 0.171 mL (n = 4).

The experimental protein content [%] was re-calculated taking the density (1.032) [4] into account in order to obtain the protein content as g/100 mL for milk. The re-calculated protein content corresponds to 3.57 g/100 mL.

Table 6: Results of the determination of nitrogen and protein in cream (declared protein content 2.5 g/100 mL).

Cream	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	2.0850	3.266	0.4158	2.65
Sample 2	1.9334	3.051	0.4173	2.66
Sample 3	2.0460	3.192	0.4136	2.64
Average [%]	-	-	0.4156	2.65
Rsd [%]	-	-	0.4	0.4

The mean blank volume (V_{Blank}) was 0.171 mL (n = 4).

The experimental protein content [%] was re-calculated taking the density (1.002) [4] into account in order to obtain the protein content as g/100 mL for cream. The re-calculated protein content corresponds to 2.66 g/100 mL.

Table 7: Results of the determination of nitrogen and protein in lassi drink (declared protein content 2.5 g/100 mL).

Lassi drink	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	2.4885	3.533	0.3784	2.41
Sample 2	2.4065	3.408	0.3768	2.40
Sample 3	2.4491	3.441	0.3740	2.39
Average [%]	-	-	0.3764	2.40
Rsd [%]	-	-	0.6	0.6

Table 8: Results of the determination of nitrogen and protein in parmesan cheese (declared protein content 33 g/100 g).

Parmesan cheese	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.4863	9.324	5.273	33.6
Sample 2	0.4507	8.612	5.247	33.5
Sample 3	0.4196	8.067	5.272	33.6
Average [%]	-	-	5.264	33.6
Rsd [%]	-	-	0.3	0.3

The mean blank volume (V_{Blank}) was 0.171 mL (n = 4).

6. Comparison to Standard Methods

This application note is based on the standard method ISO 8968-2 with minor differences. These differences are shown in Table 9.

Table 9: Differences to ISO 8968-2.

	Application note	ISO 8968-2	Notes / Impace
Sample tube	300 mL	250 mL	No impact.
Sample weight	2.5 g	5 mL	No impact, homogeneous sample.
Catalyst	2 × 3.7 g tablets. Composition 94.4% K_2SO_4 2.8% TiO_2 2.8% $CuSO_4$*$5H_2O$	12 g K_2SO_4 + 1 mL $CuSO_4$ Solution → 5g $CuSO_4$ x 5 H_2O in 100 mL water	The choice of catalyst does not influence the result. Digestion time is reduced using Titanium Tablets, see Application Note 078/2012.
Sulfuric acid	15 mL	20 mL	No impact, same ration of sulfuric acid/catalyst.
Sodium hydroxide	60 mL (Conc. 32%)	65 mL (Conc. 40%)	No impact, same ratio of sodium hydroxide/sulfuric acid.
Titration solution	H_2SO_4 0.2N	HCl 0.1N	No impact consumption of the titration solution should be between 3-17 mL.
Blanks with sucrose	No	With sucrose.	No significant difference observed between blanks with and without sucrose.

7. Conclusion

The determination of nitrogen and protein in dairy products 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 of the dairy products and with the results of the accelerated Kjeldahl method with hydrogen peroxide (see Application Note 103/2013), with low relative standard deviations (rsd). The recovery with tryptophan was 99.8% (rsd = 0.3%), which was within the specification of ≥ 98% [1,2,3].

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] ISO 8968-2:2001 Milk-Determination of nitrogen content Part 2: Block-digestion method.
[2] AOAC 991.20 Nitrogen (Total) in Milk.
[3] LFGB §64 L01.00-10/2.
[4] Kessler, H.-G.: Lebensmittel-und Bioverfahrenstechnik, Molkereitechnologie, Verlag A. Kessler, Freising, 4. Auflage 1996.

Operation Manual of KjelDigester K-446/K-449
Operation Manual of Scrubber K-415
Operation Manual of KjelMaster system K-375/K-376/K-377