Application Note No. 180/2015
Nitrogen & protein determination in milk powder

KjelDigester K-449, KjelMaster K-375 with KjelSampler K-376:
Nitrogen and Protein Determination in Milk Powder (Certified Reference Material) According to the Kjeldahl Method by Colorimetric Titration

1 Introduction

A reliable and efficient method for the determination of total nitrogen and protein in milk powder, according to EN ISO 8968-1:2014 [1], AOAC 930.29 [2] and AOAC 991.20 [3], is presented. The samples are digested using the KjelDigester K-449. The distillation and boric acid titration are performed with the KjelMaster System K-375 / K-376 equipped with a colorimetric sensor. The combination of the KjelDigester K-449 and the KjelMaster system K-375 / K-376 increases the sample throughput.

2 Equipment

- KjelDigester K-449 (the parameters used are also valid for the K-446)
- Scrubber K-415 TripleScrub ^{ECO}
- 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)

3 Chemicals and Materials

Chemicals:

- Sulfuric acid conc 98 %, Merck (1007482500)
- Titanium, BUCHI Kjeldahl Tablet (11057980)
- Sodium hydroxide 32 %, Brenntag (81980-452)
- Sher indicator, BUCHI (003512)
- Boric acid (H_3BO_3) 4 %, 400 g boric acid, Brenntag (80948-155) diluted to 10 L with deionized water, pH adjusted to 4.65, Sher indicator 2.5 mL/L
- 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

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

Sample:

- Reference material: whole milk powder with a protein content of 26.44 g/100 g
- Skimmed milk powder with a declared protein content of 33 g/100 g

The reference material was purchased at LVU, Herbolzheim (Germany).

4 Procedure

The determination of nitrogen and protein in milk powder includes the following steps:

- Digestion of the sample, using the KjelDigester K-449 or the K-446
- Distillation and colorimetric titration of the sample, using KjelMaster system K-375 / K-376

4.1 Digestion method – Reference sample

1. Preheat the KjelDigester K-449 to 350 °C
2. Place each sample in a 300 mL sample tube, the sample weight is described in Table 1

Table 1: Sample weight

Sample	Weight [g]
Whole milk powder	0.5
Simmed milk powder	0.4

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 by applying the temperature profile listed in Table 2.
7. Let the samples cool down when the digestion is completed

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

Step	Temperature [°C]	Time [min]
1	350	0
2	420	120
Cooling	–	35

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

4.2 Distillation and titration

For colorimetric titration it`s necessary to determine the setpoint of the boric acid solution in advance to the blank and sample determinations. It is necessary to determine the setpoint every day before starting sample determinations, and when the method is changed or fresh chemicals are used to adjust the device to the current conditions.
The detailed procedure including the preparation of the sensor is described in the Technical Note 179/2015 “Colorimetric titration procedure using Sher indicator” [5].
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 paramters 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 method from the list

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 al residues from over titration from the receiving vessel
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 1	788.9 mV
Setpoint 2	807.2 mV
Deviation	18.3 mV

Table 5: 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	Colorimetric
Reaction time	5 s	Titration mode	Online
Distillation mode	Fixed time	Titration start time	90 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	Optimal
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.

4.3 Calculation

The results are calculated as a percentage of nitrogen in the sample. 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.

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

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

$$\%P_{Sample} = w_N \cdot PF \cdot 100\ \% \quad (3)$$

$$\%\,Pr_{\,rec\ rate} = \frac{\%P_{Sample} \bullet 100}{\%P_{reference}} \quad (4)$$

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

$\%Pr_{\,rec\ rate}$: recovery rate [%]

$\%P_{sample}$: percentage of weight of protein [%]

PF : sample-specific protein factor (6.38 for dairy products)

5 Results

The measured nitrogen and protein contents in whole milk powder and skimmed milk powder are shown in Table 6 and 7.

Table 6: Measured nitrogen and protein contents in whole milk powder (labelled protein content 26.44 g/100 g)

Whole milk powder	m_{Sample} [g]	$V_{Titrant}$ [mL]	%N	%P	Recovery rate [%]
Sample 1	0.5252	7.940	4.115	26.255	99.3
Sample 2	0.5384	8.114	4.105	26.189	99.1
Sample 3	0.5082	7.701	4.121	26.293	99.4
Sample 4	0.5207	7.843	4.099	26.149	98.9
Sample 5	0.5479	8.309	4.133	26.371	99.7
Average [%]	-	-	4.115	26.251	99.3
Rsd [%]	-	-	0.297	0.297	-

The mean blank volume (V_{Blank}) was 0.225 mL (n = 5).

The whole milk powder was purchased at LVU, Herbolzheim (Germany). It has a declared protein content of 26.44 % ±0.640, the protein content was verified by an interlaboratory test. We measured a protein content of 26.25 % confirming the labelled content and viability of the Kjeldahl method.

Table 7: Measured nitrogen and protein contents in whole milk powder (labelled protein content 33 g/100 g)

Whole milk powder	m_{Sample} [g]	$V_{Titrant}$ [mL]	%N	%P	Recovery rate [%]
Sample 1	0.4065	7.785	5.210	33.240	100.7
Sample 2	0.4208	8.127	5.261	33.563	101.7
Sample 3	0.4025	7.704	5.205	33.211	100.6
Sample 4	0.4102	7.861	5.215	33.271	100.8
Sample 5	0.4222	8.068	5.204	33.202	100.6
Average [%]	-	-	5.219	33.297	100.9
Rsd [%]	-	-	0.405	0.405	-

The mean blank volume (V_{Blank}) was 0.225 mL (n = 5).
The measured protein content of 33.3 % corresponds well with the labelled protein content of 33 g/100 g

6 Comparison to Standard Methods

This application note is based on the standard method EN ISO 8968-1:2014 (block digestion method), AOAC 930.29 and AOAC 991.20 (Block Digestor / Steam Distillation Method) with minor differences. These differences are shown in Table 8.

Table 8: Differences to EN ISO 8968-1:2014, AOAC 930.29 and AOAC 991.20

	Application note	EN ISO 8968-1:2014	AOAC 930.29 AOAC 991.20	Notes / Impact
Sample tube	300 mL	250 mL	250 mL	No impact Less problems with foaming samples. No cross contamination.
Digester	Block digester with time and temperature control	Block digester with adjustable temperature control	Block digester with adjustable temperature control	Time / temperature control is more convienient and allows for full automation.
Sample size	0.4 - 0.5 g	0.5 g ±0.05 g	1 g	No impact, consumption of the titration solution should be between 3-17 mL.
Catalyst	2 × 3.7 g Tablets Composition: 94.4 % K_2SO_4 2.8 % TiO_2 2.8 % $CuSO_4$* $5H_2O$	2 × 3.5 g K_2SO_4 0.105 g $CuSO_4$* 5 H_2O 0.105 g TiO_2	12.00 g K_2SO_4 1 mL of copper catalyst solution (0.05 g $CuSO_4$* 5 H_2O per 1 mL H_2O)	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	12 mL	20 mL	No impact, same ratio of sulfuric acid/catalyst.
Digestion time	2 h	1.75 – 3 h	1.75 - 2.5 h	No impact
Sodium hydroxide	60 mL (Conc. 32 %)	55 - 65 mL (Conc. 40 or 50 %)	55 - 65 mL (Conc. 40 or 50 %)	No impact. Comparable ratio of sodium hydroxide/sulfuric acid.
Water	50 mL	85 mL	85 mL	Less water required due to the steam transfer principle of the KjelMaster system K-375 / K-376
Boric acid solution	60 mL (Conc. 4 %)	50 mL (Conc. 4 %)	50 mL (Conc. 4 %)	Higher capacity to bind more NH_3
Distillation time	180 seconds	Until 150 mL distillate is received	Until 150 mL distillate and 200 mL total receiver volume	No impact. Distillation time must be sufficient to transfer all NH_3 into the receiving vessel.
Titration solution	H_2SO_4 0.2N	HCl 0.1N	HCl 0.1N	No impact, consumption of the titration solution should be between 3-17 mL.
Blanks with sucrose	No sucrose	With sucrose	With sucrose	No significant difference observed between the blanks.
Titration	Colorimetric (automatic titration)	Colorimetric (visible color change) or potentiometric; to the first trace of pink or pH 4.6	Visible color change; to first trace of pink	No impact, easier handling when working with automatic titration.
Indicator	Sher mixed indicator	Methyl red / bromocresol green 1:5	Methyl red / bromocresol green 1:5	No impact. With the Sher mixed indicator the color changes more sharply at pH 4.65 than methyl red / bromocresol green indicator.

7 Conclusion

The determination of nitrogen and protein in milk powder using the KjelDigester K-449 and KjelMaster system K-375 / K-376 by colorimetric titration provides reliable and reproducible results and is fully automated. These results correspond well to the labelled values of the milk powders and with the results determined by potentiometric titration shown in the Application Note 104/2013 with low relative standard deviations (rsd).
With the KjelDigester K-449 the digestion process (including preheating, digestion and cooling) is 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 allows unattended operation.

8 References

[1] EN ISO 8968-1:2014 Milk and milk products – Determination of nitrogen content - Part 1: Kjeldahl principle and crude protein calculation - Block-digestion method
[2] AOAC 991.20 Nitrogen (Total) in Milk – Kjeldahl Method - Block Digestor / Steam Distillation Method
[3] AOAC 930.29 Protein in Dried Milk

[4] Application Note 104/2013, Nitrogen and Protein Determination in Milk Powder
[5] Technical Note 179/2015 Colorimetric titration procedure using Sher indicator

KjelOpimizer App

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