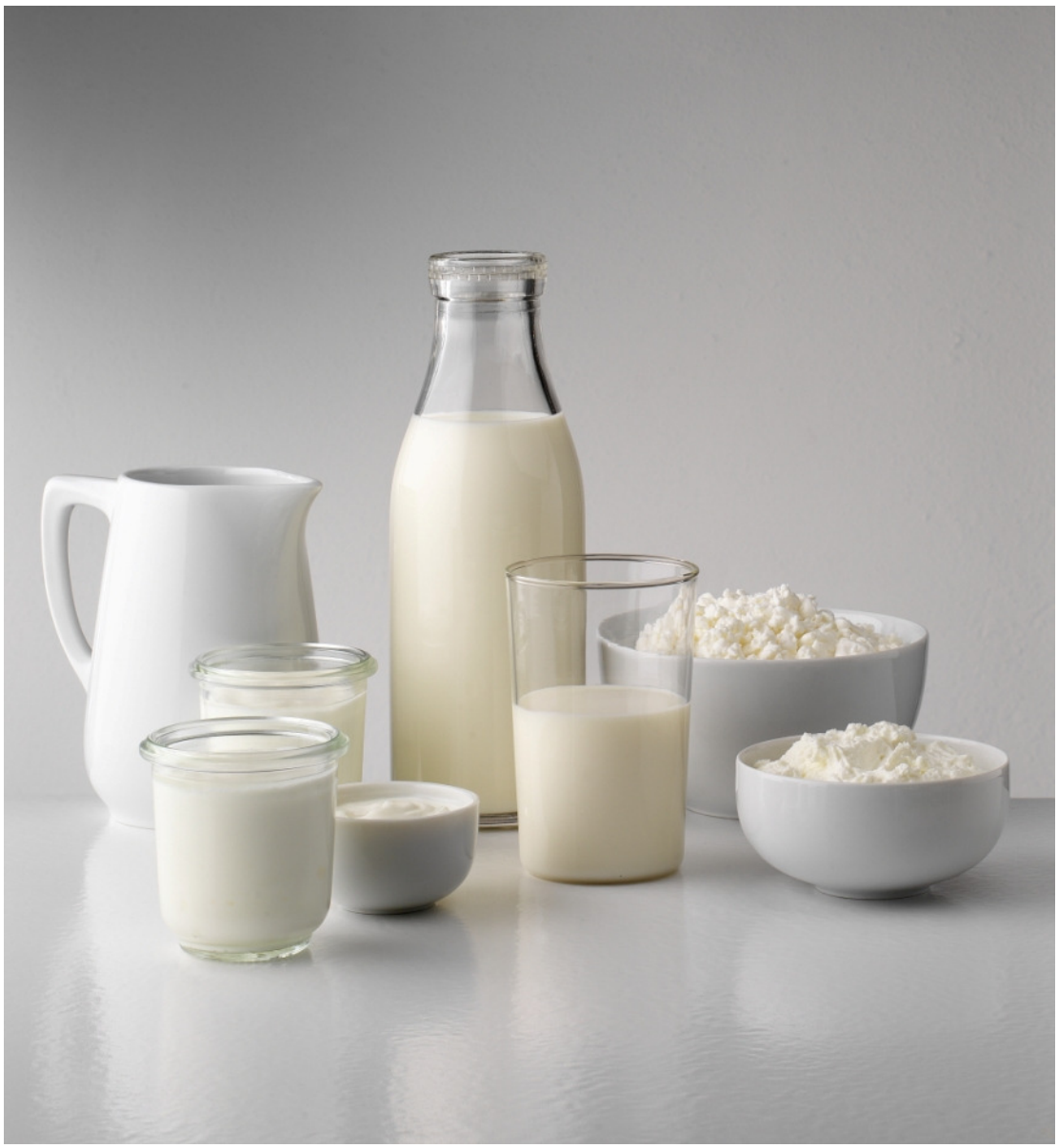
Application Note

028/2010



SpeedDigester K-436, K-439 / KjelFlex K-360

Nitrogen and Protein Determination in Whey Protein Concentrates and Demineralized Whey Powder according to the Kjeldahl Method

028/2010
SpeedDigester K-436 / K-439
KjelFlex K-360

SHORT NOTE

Nitrogen and Protein Determination in Whey Protein Concentrates and Demineralized Whey Powder according to the Kjeldahl Method

The determination of protein in food is a routine procedure for quality assurance and labelling. A simple and fast procedure for protein determination in whey protein concentrates and demineralized whey powder according to the Kjeldahl method, as described in the AOAC 991.20, LFGB §64 L01.00-10/1, and DIN EN ISO 8968-1:2001 regulations, is introduced below. The sample is digested with sulphuric acid using the SpeedDigester K-436 or K-439, followed by distillation and titration with the KjelFlex K-360. The determined protein contents correspond to the labelled values.

Introduction

Protein determination is one of the key analyses performed in the food industry. The samples require digestion with sulfuric acid to convert nitrogen into ammonium sulfate. After conversion to ammonia through the alkalinization with sodium hydroxide, the sample is distilled into a boric acid receiver by steam distillation, followed by a titration with sulfuric acid solution. The nitrogen content is multiplied by a sample-specific factor (6.38 for whey protein) to obtain the protein content.

Experimental

Instrumentation: SpeedDigester K-436, K-439, KjelFlex K-360

Samples: Whey protein concentrates, declared protein contents 70 % and 80 % in DS[1)], respectively and demineralized whey powder, declared protein content min. 12 % in DS.

Figure 1: Whey protein concentrates 80 (left) and 70 (right)

Determination: Approx. 0.15 – 0.65 g of the samples were added directly into a sample tube. A portion of 20 ml of sulfuric acid and 2 Kjeldahl tablets were added, and the digestion was performed using the "whey powder" method (K-439) or the parameters specified in Table 1. After digestion, the ammonia of the sample was distilled into a boric acid solution by steam distillation and titrated with sulfuric acid (Table 2).

The method was verified by using 0.18 g tryptophan as the reference substance.

Table 1: Temperature profile for digestion with the K-436, K-439

	K-439		K-436	
Step	Temp. [°C]	Time [min]	Level	Time [min]
Preheat	480	-	8.5	10
1	480	10	8.5	10
2	550	10	9.5	15
3	490	65	8.5	75
Cooling	-	30	-	30

[1)]DS = dry substance

Table 2: Parameters for distillation with the KjelFlex K-360 and titration

KjelFlex K-360		Metrohm 848 Titrino plus	
Water	80 ml	Titration Solution	H_2SO_4 0.1 mol/l
NaOH	80 ml	Endpoint	pH 4.65
Boric acid 4 %	50 ml	Titration Rate	Optimal
Reaction Time	5 s	Stop Crit.	Drift
Steam Power	100 %	Stop Drift	20 µl/min
Dist. Time	240 s	Stop Volume	40 ml
Titration Start	240 s	Stop Time	Off
Titration Type	Boric Acid	Filling Rate	Max. ml/min
Stirrer Sp. Dist.	5		
Stirrer Sp. Titr.	7		

Results

The tryptophan recoveries were 99.5 %, rsd 0.36 % (K-439) and 99.4 %, rsd 0.41 % (K-436).

The determined protein contents are presented in Table 3.

Table 3: Determined protein contents in WPC and whey powder (relative standard deviation in brackets, n=4)

	Protein content K-439 [%]	Protein content K-436 [%]
WPC 70	71.55 (0.18 %)	71.35 (0.20 %)
WPC 80	80.12 in DS (0.11 %)	80.10 in DS (0.23 %)
Demineralized whey powder 90	13.88 in DS (0.32 %)	13.88 in DS (0.25 %)

Conclusion

The determination of protein contents in WPC and whey powder according to Kjeldahl using SpeedDigester K-436, K-439, and KjelFlex K-360 provides reliable and reproducible results that correspond to the expected values with low relative standard deviations. The total digestion time is approx. 85 min (K-439) or 100 min (K-436).

References

DIN EN ISO 8968:2001
LFBG §64 L01.00-10/1
AOAC 991.20 Part "Traditional Method"
Operation manual SpeedDigester K-425 / K-436
Operation manual SpeedDigester K-439
Operation manual KjelFlex K-360
For more detailed information please refer to Application Note 028/2010

1 Introduction

An easy and reliable method for the determination of total nitrogen and protein in whey protein concentrates and demineralized whey powder according to the Kjeldahl method, as described in the AOAC 991.20, LFGB §64 L01.00-10/1, and DIN EN ISO 8968-1:2001 regulations, is introduced below. The samples are digested using the SpeedDigester K-436 and K-439. The distillation and boric acid titration is performed with the KjelFlex K-360 and Metrohm 848 Titrino Plus.

2 Equipment

- SpeedDigester K-436, K-439 (the parameters used for K-436 are also valid for Büchi SpeedDigester K-425)
- Scrubber B-414 with condenser
- KjelFlex K-360 with titration set (or any other Büchi Kjeldahl distillation unit)
- 848 Titrino Plus (Metrohm)
- Connecting cable from Titrator to K-360 (Büchi No. 11055333)
- Analytical balance (accuracy ± 0.1 mg)

3 Chemicals and Materials

- Sulfuric acid conc 98 %, Fluka (84727)
- Catalyst, Hg/Se-free, Büchi (028765)
- Sodium hydroxide 32 %, Brenntag (81980-452)
- Boric acid 4 %, 200 g boric acid, Brenntag (80948-155) diluted to 5 l with deionized water, pH adjusted to 4.65
- Sulfuric acid 0.1 mol/l, Riedel de Haën (35357) standard solution
- 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
- Sucrose, Riedel-de Haën (16104)
- DL-tryptophan, Fluka (93680; assay: > 99 %)

4 Samples

- WPC 70, declared protein content 70 %
- WPC 80, declared protein content 80 % in DS
- Demineralized whey powder, declared protein content min 12 % in DS

5 Procedure

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

- Homogenization of the sample
- Digestion of the sample, using SpeedDigester K-436, K-439
- Distillation of the sample, using KjelFlex K-360
- Titration with Metrohm 848 Titrino Plus

5.1 Digestion method - tryptophan (verification of the method)

- Place approx. 0.18 g tryptophan in a 300 ml sample tube
- Add 0.67 g sucrose, 2 Kjeldahl tablets and a portion of 20 ml of sulfuric acid (98 %)
- Prepare additional blanks, chemicals without sample
- Carefully suspend the sample by gently swirling the tube
- Connect the Scrubber B-414 to the SpeedDigester K-436 or K-439 for absorbing the acid fumes created during digestion
- Insert the rack containing the samples into the preheated unit
- Digest the samples according to the “whey powder” method (K-439) or the parameters listed in Table 1

5.2 Digestion method samples

- Place approx. 0.15 – 0.65 g of the sample (depends on concentration of protein and organic matrix) in a 300 ml sample tube
- Add 2 Kjeldahl tablets and a portion of 20 ml of sulfuric acid (98 %)
- Prepare additional blanks, chemicals without sample
- Carefully suspend the sample by gently swirling the tube
- Connect the Scrubber B-414 to the SpeedDigester K-436 or K-439 for absorbing the acid fumes created during digestion
- Insert the rack containing the samples into the preheated unit
- Digest the samples according to the “whey powder” method (K-439) or the parameters listed in Table 1

Table 1: Temperature profile for digestion with the K-436, K-439

	K-439		K-436	
Step	Temperature [°C]	Time [min]	Heating Level	Time [min]
Preheating	480	-	8.5	10
1	480	10	8.5	10
2	550	10	9.5	15
3	490	65	8.5	75
Cooling	-	30	-	30

- If the liquid inside the sample tube is not clear and blue-green, digest for an additional 30 min as described in step 3
- Let the samples cool down to ambient temperature

NOTE: When the samples are placed in the cooling position it takes approx. 30 min to cool them down; when they are left in the heating chamber it takes at least 60 min.

5.3 Distillation and titration

Distill the samples according to the parameters listed in Table 2. The determination was carried out with a KjelFlex K-360 connected to a Metrohm 848 Titrino Plus.

Table 2: Distillation and titration parameters

KjelFlex K-360		Metrohm 848 Titrino plus	
Water	80 ml	Titration Solution	H_2SO_4 0.1 mol/l
Sodium Hydroxide	80 ml	Endpoint	pH 4.65
Boric Acid 4 %	50 ml	Titration Rate	optimal
Reaction Time	5 s	Stop Criterion	Drift
Steam Power	100 %	Stop Drift	20 µl/min
Distillation Time	240 s	Stop Volume	40 ml
Titration Start	240 s	Stop Time	Off
Titration Type	Boric Acid	Filling Rate	max. ml/min
Stirrer Speed Dist.	5		
Stirrer Speed Titr.	7		

5.4 Calculation

The results are calculated as 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.

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

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

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

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] (recovery: consider the assay of tryptophan)
1000	: conversion factor [ml/l]
%N	: percentage of weight of nitrogen
%P	: percentage of weight of protein
PF	: sample-specific protein factor (6.38 for WPC and whey powder)

6 Results

6.1 Digestion with SpeedDigester K-439

6.1.1 Recovery of tryptophan

The results of the nitrogen determination and recovery in tryptophan are presented in Table 3. The nominal value of tryptophan (assay: ≥ 99 %) is 13.58 % nitrogen. The recoveries are within the specification of ≥ 98 % [1], [2].

Table 3: Results for the recovery of nitrogen in tryptophan with K-439

Tryptophan	m_{Sample} [g]	V_{Sample} [ml]	%N	Recovery [%]
Sample 1	0.1827	9.086	13.62	100.3
Sample 2	0.1798	8.890	13.54	99.7
Sample 3	0.1812	8.947	13.52	99.6
Sample 4	0.1784	8.791	13.49	99.3
Sample 5	0.1789	8.825	13.50	99.4
Sample 6	0.1783	8.791	13.50	99.4
Sample 7	0.1818	8.964	13.50	99.4
Sample 8	0.1803	8.863	13.46	99.1
Average	**-**	**-**	**13.52**	**99.5**
Rsd [%]	**-**	**-**	**0.36**	**0.36**

The mean blank volume for this sample was 0.202 ml (n = 4).

6.1.2 Protein determination in whey protein concentrates and whey powder

The results of the determination of nitrogen in whey protein concentrates and whey powder are presented in Tables 4 - 6.

Table 4: Results for the determination of nitrogen in WPC 70 with K-439 (declared protein content 70 %)

WPC 70	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	0.1536	6.340	11.20	71.45
Sample 2	0.1550	6.402	11.21	71.52
Sample 3	0.1483	6.132	11.21	71.50
Sample 4	0.1478	6.132	11.24	71.74
Average	**-**	**-**	**11.22**	**71.55**
Rsd [%]	**-**	**-**	**0.18**	**0.18**

The mean blank volume for this sample was 0.200 ml (n = 4).

Table 5: Results for the determination of nitrogen in WPC 80 with K-439 (declared protein content 80 % in DS)

WPC 80	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	0.1422	6.238	11.90	75.90
Sample 2	0.1481	6.492	11.90	75.94
Sample 3	0.1542	6.746	11.89	75.88
Sample 4	0.1499	6.552	11.87	75.75
Average	**-**	**-**	**11.89**	**75.87**
Rsd [%]	**-**	**-**	**0.11**	**0.11**

The mean blank volume for this sample was 0.199 ml (n = 4).
The experimental protein content was corrected for a dry substance through calculation taking the moisture content (5.3%) into account. The protein content corrected for moisture = 80.12 %.

Table 6: Results for the determination of nitrogen in whey powder with K-439 (declared protein content >12 % in DS)

Whey powder	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	0.6251	4.904	2.11	13.45
Sample 2	0.5898	4.672	2.12	13.55
Sample 3	0.6018	4.754	2.12	13.53
Sample 4	0.6129	4.834	2.12	13.51
Average	**-**	**-**	**2.12**	**13.51**
Rsd [%]	**-**	**-**	**0.32**	**0.32**

The mean blank volume for this sample was 0.200 ml (n = 4).
The experimental protein content was corrected for a dry substance through calculation taking the moisture content (2.7%) into account. The protein content corrected for moisture = 13.88 %.

6.2 Digestion with SpeedDigester K-436

6.2.1 Recovery of tryptophan

The results of the recovery of nitrogen in tryptophan are presented in Table 7. The nominal value of tryptophan (assay: ≥ 99 %) is 13.58 % nitrogen. The determined values lie within the specification of ≥ 98 % [1], [2].

Table 7: Results for the recovery of nitrogen in tryptophan with K-436

Tryptophan	m_{Sample} [g]	V_{Sample} [ml]	%N	Recovery [%]
Sample 1	0.1820	8.945	13.50	99.4
Sample 2	0.1794	8.811	13.48	99.3
Sample 3	0.1819	8.960	13.53	99.6
Sample 4	0.1798	8.811	13.45	99.1
Sample 5	0.1800	8.774	13.38	98.5
Sample 6	0.1780	8.780	13.54	99.7
Sample 7	0.1781	8.788	13.54	99.7
Sample 8	0.1766	8.697	13.52	99.5
Average	**-**	**-**	**13.49**	**99.4**
Rsd [%]	**-**	**-**	**0.41**	**0.41**

The mean blank volume for this sample was 0.177 ml (n = 4).

6.2.2 Protein determination in whey protein concentrates and whey powder

The results of the determination of nitrogen in whey protein concentrates and whey powder are presented in Tables 8 - 10.

Table 8: Results for the determination of nitrogen in WPC 70 with K-436 (declared protein content 70 % in DS)

WPC 70	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	0.1523	6.274	11.18	71.35
Sample 2	0.1464	6.022	11.15	71.15
Sample 3	0.1410	5.832	11.20	71.47
Sample 4	0.1709	7.024	11.20	71.43
Average	**-**	**-**	**11.18**	**71.35**
Rsd [%]	**-**	**-**	**0.20**	**0.20**

The mean blank volume for this sample was 0.194 ml (n = 4).

Table 9: Results for the determination of nitrogen in WPC 80 with K-436 (declared protein content 80 % in DS)

WPC 80	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	0.1455	6.390	11.91	75.97
Sample 2	0.1462	6.418	11.90	75.95
Sample 3	0.1511	6.620	11.89	75.87
Sample 4	0.1518	6.626	11.85	75.59
Average	**-**	**-**	**11.89**	**75.85**
Rsd [%]	**-**	**-**	**0.23**	**0.23**

The mean blank volume for this sample was 0.206 ml (n = 4).

The experimental protein content was corrected for a dry substance through calculation taking the moisture content (5.3 %) into account. The protein content corrected for moisture = 80.10 %.

Table 10: Results for the determination of nitrogen in whey powder 90 with K-439 (declared protein content >12 % in DS)

Whey powder 90	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	0.6077	4.788	2.12	13.51
Sample 2	0.6114	4.832	2.13	13.56
Sample 3	0.6222	4.892	2.12	13.50
Sample 4	0.6134	4.820	2.11	13.48
Average	**-**	**-**	**2.12**	**13.51**
Rsd [%]	**-**	**-**	**0.25**	**0.25**

The mean blank volume for this sample was 0.194 ml (n = 4).

The experimental protein content was corrected for a dry substance through calculation taking the moisture content (2.7 %) into account. The protein content corrected for moisture = 13.88 %.

7 Comparison to Standard Methods

The standard methods DIN EN ISO 8968-1:2001 and LFGB §64 L01.00-10/1 are identical. AOAC 991.20 Part “Traditional Method” does not use sucrose and water as blanks. All other parts are identical with DIN EN ISO 8968-1:2001 and LFGB §64 L01.00-10/1.

Table 11: Differentiation to the standard methods

	This application note	Standard methods	Notes/Impact
Sample tube	300 ml	500 – 800 ml	No impact
Catalyst	10 g Tablets cont. - 47.7 % K_2SO_4; - 47.7 % Na_2SO_4; - 2.8 % TiO_2; - 1.8 % $CuSO_4$	15 g K_2SO_4 + 1 ml $CuSO_4$ Solution[1)]	Easy to handle especially in routine analytics. The choice of catalyst does not influence the result.
Sulfuric acid	20 ml	25 ml	No impact. Same ratio of sulfuric acid / catalyst
Water	80 ml	300 – 400 ml	The K-360 generates steam in a separate vessel; therefore it is not necessary to add such a high amount of water to the digested sample as described in the standard method.
Sodium hydroxide	90 ml (Conc.: 32 %)	75 ml (Conc.: 50 %)	No impact. Same ratio of sodium hydroxide / sulfuric acid. Sodium hydroxide 32% is more gentle to the pump than higher concentrated alkali.
Blank with sucrose for determination of samples	no	DIN EN ISO and LFGB §64 use 0.85 g and 5 ml water for blank additionally to the chemicals.	No significant difference observed between blanks with and without sucrose. For recovery of tryptophan, sucrose was added to the blanks.

[1)] 5 g $CuSO_4$ x 5 H_2O diluted to 100 ml deionized Water

8 Conclusion

The determination of nitrogen and protein in whey protein concentrates using the SpeedDigester K-436, K-439, and KjelFlex K-360 provides reliable and reproducible results that correspond to the labelled values with low relative standard deviations. There are no differences between the results obtained with the K-436 and the K-439 respectively. The digestion time is very fast: 85 min for the K-439 and 100 min for the K-436. The recoveries with tryptophan were 99.5 % (K-439) and 99.4 % (K-436) respectively and are within the specification of ≥98 %.

9 References

[1] DIN EN ISO 8968-1:2001
[2] LFGB §64 L01.00-10/1
AOAC 991.20 Part “Traditional Method”

Operation manual of SpeedDigester K-425 / K-436
Operation manual of SpeedDigester K-439
Operation manual of Scrubber B-414
Operation manual of KjelFlex K-360
Operation manual of 848 Titrino Plus (Metrohm)

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