Application Note

038/2010

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

Nitrogen and Protein Determination in Pasta according to the Kjeldahl Method (Back Titration)

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

SHORT NOTE

Nitrogen and Protein Determination in Pasta according to the Kjeldahl Method (Back Titration)

The determination of protein in food is a routine procedure for quality assurance and labelling. A simple and fast procedure for protein determination in pasta, as described in the AOAC 930.25, is introduced below. The sample is digested with sulfuric acid using the SpeedDigester K-436 or K-439, followed by distillation and back 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 sulfuric acid receiver by steam distillation, followed by a back titration with sodium hydroxide solution. The nitrogen content is multiplied by a sample-specific factor (6.25 for pasta) to obtain the protein content.

Experimental

Instrumentation: SpeedDigester K-436, K-439, KjelFlex K-360, external dosage device, 848 Titrino plus

Samples:

Macaroni and tortellinis, labelled protein content 12 % and 15 %, respectively.

Figure 1: Macaroni (left) and tortellinis (right)

Determination: Approx. 2 - 3.5 g of the samples (depends on concentration of protein and organic matrix) were added directly into a sample tube. A portion of 25 ml of sulfuric acid and 2 Kjeldahl tablets were added, and the digestion was performed using the "pasta" method (K-439) or the parameters specified in Table 1. After digestion, the ammonia of the sample was distilled into a sulfuric acid solution by steam distillation and back titrated with sodium hydroxide (Table 2).

The method was verified by using 0.25 g glycine 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

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

KjelFlex K-360		Metrohm 848 Titrino plus	
Water	50 ml	Receiving solution[1)]	20.0 ml H_2SO_4 0.25 mol/l
Sodium hydroxide	90 ml	Titration Solution	NaOH 0.5 mol/l
Reaction Time	5 s	Endpoint	pH 4.65
Steam Power	100 %	Titration Rate	Optimal
Dist. Time	240 s	Stop Crit.	Drift
Titration Start	240 s	Stop Drift	20 µl/min
Titration Type	Back Titration	Stop Volume	40 ml
Stirrer Sp. Dist.	5	Stop Time	Off
Stirrer Sp. Titr.	7	Filling Rate	Max. ml/min

[1)] The volume of the receiving solution must be entered at the external dosage device.

Results

The glycine recoveries were 99.6 %, rsd 0.35 % (K-439) and 99.7 %, rsd 0.21 % (K-436).

The determined protein contents are presented in Table 3.

Table 3: Determined protein contents in macaroni products (relative standard deviation in brackets, n=4)

	Protein content K-439 [%]	Protein content K-436 [%]
Elbow pasta	13.50 (0.32 %)	13.61 (0.30 %)
Tortellini	14.31 (0.63 %)	14.36 (0.35 %)

Conclusion

The determination of protein contents in macaroni products 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

AOAC 930.25

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 038/2010

1 Introduction

An easy and reliable method for the determination of total nitrogen and protein in macaroni products according to the Kjeldahl method, as described in the AOAC 930.25 regulations, is introduced below. The samples are digested using the SpeedDigester K-436 and K-439. The distillation and back titration is performed with the KjelFlex K-360, external dosage device 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)
- External dosage device (Schott Titronic universal) (Büchi No. 043596)
- Connecting cable from dispenser (Schott) to K-360 (Büchi No. 043621)
- 848 Titrino plus (Metrohm)
- Connecting cable from Titrator (Metrohm) to K-360 (Büchi No. 11055333)
- Analytical balance (accuracy ± 0.1 mg)
- Grindomix GM200 (Retsch)

3 Chemicals and Materials

- Sulfuric acid conc 98 %, Fluka (84727)
- Catalyst, Hg/Se-free, Büchi (028765)
- Sodium hydroxide 32 %, Brenntag (81980-452)
- Sulfuric acid 0.25 mol/l, Merck (1.09073.1000) standard solution
- Sodium hydroxide 0.5 mol/l, Merck (1.09138.1000) 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
- Glycine, Sigma-Aldrich (33226; assay: 99.7 %)

4 Samples

- Macroni, labelled protein content 12 %
- Tortellini, labelled protein content 15 %

The samples were purchased at a local supermarket.
The samples were mixed twice for 20 s (10'000 rpm) up to visual homogeneity.

5 Procedure

The determination of nitrogen and protein in shortbread 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
- Back titration with Metrohm 848 Titrino Plus

5.1 Digestion method - glycine (verification of the method)

- Place approx. 0.25 g glycine 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 “pasta” method (K-439) or the parameters listed in Table 1

5.2 Digestion method samples

- Place approx. 2 – 3.5 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 25 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 “pasta” 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 an external dosage device and a Metrohm 848 Titrino plus.

Table 2: Distillation and titration parameters

KjelFlex K-360		Metrohm 848 Titrino plus	
Water	50 ml	Receiving solution[1)]	20.0 ml H_2SO_4 0.25 mol/l
Sodium Hydroxide	90 ml	Titration Solution	NaOH 0.5 mol/l
Reaction Time	5 s	Endpoint	pH 4.65
Steam Power	100 %	Titration Rate	optimal
Distillation Time	240 s	Stop Criterion	Drift
Titration Start	240 s	Stop Drift	20 µl/min
Titration Type	Back titration	Stop Volume	40 ml
Stirrer Speed Dist.	5	Stop Time	Off
Stirrer Speed Titr.	7	Filling Rate	max. ml/min

[1)] The external dosage device is connected to the port for the manual key button. The volume of the receiving solution must be entered at the external dosage device. For this purpose, refer to the Operation Manual of the dosage device.

Configuration K-360: Signal “ready”: No
Signal “active”: No
Signal “end”: 40 s

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.

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

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

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

w_N : weight fraction of nitrogen

V_{Blank} : mean amount of titrant for the blank [ml]

V_{Sample} : amount of sodium hydroxide titrant for the sample [ml]

c : titrant concentration [mol/l]

f : factor of the titrant (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 glycine)

1000 : conversion factor [ml/l]

%N : percentage of weight of nitrogen

%P : percentage of weight of protein

PF : sample-specific protein factor (6.25 for pasta)

6 Results

6.1 Digestion with SpeedDigester K-439

6.1.1 Recovery of glycine

The results of the nitrogen determination and recovery in glycine are presented in Table 3. The nominal value of glycine (assay: 99.7 %) is 18.60 % nitrogen. The recoveries are within the specification of 98 – 102 % [1].

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

Glycine	m_{Sample} [g]	V_{Sample} [ml]	%N	Recovery [%]
Sample 1	0.2607	13.028	18.62	100.1
Sample 2	0.2348	13.754	18.51	99.5
Sample 3	0.2339	13.764	18.55	99.7
Sample 4	0.2674	12.908	18.47	99.3
Average	**-**	**-**	**18.54**	**99.6**
Rsd [%]	**-**	**-**	**0.35**	**0.35**

The mean blank volume for this sample was 19.959 ml (n = 4).

6.1.2 Protein determination in pasta

The results of the determination of nitrogen in pasta are presented in Tables 4 - 5.

Table 4: Results for the determination of nitrogen in macaroni with K-439 (labelled protein content 12 %)

Macaroni	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	3.4857	9.234	2.164	13.52
Sample 2	3.5748	8.962	2.163	13.52
Sample 3	3.5186	9.136	2.163	13.52
Sample 4	3.5176	9.208	2.149	13.43
Average	**-**	**-**	**2.160**	**13.50**
Rsd [%]	**-**	**-**	**0.32**	**0.32**

The mean blank volume for this sample was 20.004 ml (n = 4).

Table 5: Results for the determination of nitrogen in tortellini with K-439 (labelled protein content 15 %)

Tortellini	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	1.9618	13.512	2.305	14.41
Sample 2	2.0798	13.196	2.281	14.25
Sample 3	2.0824	13.208	2.274	14.21
Sample 4	2.0684	13.186	2.297	14.35
Average	**-**	**-**	**2.289**	**14.31**
Rsd [%]	**-**	**-**	**0.63**	**0.63**

The mean blank volume for this sample was 19.969 ml (n = 4).

6.2 Digestion with SpeedDigester K-436

6.2.1 Recovery of glycine

The results of the nitrogen determination and recovery in glycine are presented in Table 6. The nominal value of glycine (assay: 99.7 %) is 18.60 % nitrogen. The recoveries are within the specification of 98 - 102 % [1].

Table 6: Results for the recovery of nitrogen in glycine with K-436

Glycine	m_{Sample} [g]	V_{Sample} [ml]	%N	Recovery [%]
Sample 1	0.2529	13.266	18.54	99.6
Sample 2	0.2607	13.070	18.51	99.5
Sample 3	0.2559	13.164	18.60	100.0
Sample 4	0.2660	12.922	18.53	99.6
Average	**-**	**-**	**18.54**	**99.7**
Rsd [%]	**-**	**-**	**0.21**	**0.21**

The mean blank volume for this sample was 19.959 ml (n = 4).

6.2.2 Protein determination in pasta

The results of the determination of nitrogen in pasta are presented in Tables 7 - 8.

Table 7: Results for the determination of nitrogen in macaroni with K-436 (labelled protein content 12 %)

Macaroni	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	3.5703	8.932	2.172	13.57
Sample 2	3.6044	8.812	2.175	13.59
Sample 3	3.5178	9.020	2.187	13.67
Sample 4	3.5261	9.032	2.179	13.62
Average	**-**	**-**	**2.178**	**13.61**
Rsd [%]	**-**	**-**	**0.30**	**0.30**

The mean blank volume for this sample was 20.004ml (n = 4).

Table 8: Results for the determination of nitrogen in tortellini with K-436 (labelled protein content 15 %)

Tortellini	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	2.0931	13.134	2.287	14.29
Sample 2	2.0108	13.382	2.294	14.34
Sample 3	2.0535	13.216	2.303	14.39
Sample 4	2.0960	13.074	2.304	14.40
Average	**-**	**-**	**2.297**	**14.36**
Rsd [%]	**-**	**-**	**0.35**	**0.35**

The mean blank volume for this sample was 19.969 ml (n = 4).

7 Comparison to Standard Methods

This application note is based on the standard method AOAC 930.25.

Table 7: Differentiation to the standard method

	This application note	Standard method	Notes/Impact
Sample weight	2 – 3.5 g	0.7 – 2.2 g	In back titration higher contents of nitrogen are needed to obtain a reasonable difference in titrant consumption between blanks and samples.
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 + 0.7 g HgO	Easy to handle especially in routine analytics. The choice of catalyst does not influence the result. No toxic Hg.
Water	50 ml	200 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.

8 Conclusion

The determination of nitrogen and protein in macaroni products using the SpeedDigester K-436, K-439, and KjelFlex K-360 provides reliable and reproducible results that correspond to the labelled value 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 glycine were 99.6 % (K-439) and 99.7 % (K-436) respectively and are within the specification of 98 – 102 %.

9 References

[1] Application Note 001-437_370-03C: Operational Quality Check Procedure

AOAC 930.25

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 external dosage device (Schott)
Operation manual of 848 Titrino plus (Metrohm)

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