Application Note

046/2010

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

Nitrogen and Protein Determination in Juice and Lassi according to the Kjeldahl Method

Nitrogen and Protein Determination in Juice and Lassi 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 juice and lassi according to the Kjeldahl method, as described in the Swiss Food Manual 22/4.1 – 4.2 and other regulations, is introduced below. The sample is digested with sulfuric acid using the SpeedDigester K-436 or K-439, followed by distillation and titration with the KjelFlex K-360. There are no differences between the results obtained with the K-436 and the K-439 respectively. The protein contents are reliable and reproducible with low relative standard deviations.

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 factor (general factor for food: 6.25) to obtain the protein content.

Experimental

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

Samples: Fruit juice, beetroot juice and India lassi.

Figure 1: Fruit juice, India lassi and beetroot juice.

Determination: 5 – 8 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 "beverages" 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

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

KjelFlex K-360		Schott TitroLine easy	
Water	80 ml	Titration Solution	H_2SO_4 0.1 mol/l
NaOH	80 ml	Internal Method	pH fast strong
Boric acid 4 %	50 ml	Endpoint	pH 4.6
Reaction Time	5 s		
Steam Power	100 %		
Dist. Time	240 s		
Titration Start	240 s		
Titration Type	Boric Acid		
Stirrer Sp. Dist.	5		
Stirrer Sp. Titr.	7		

Results

The tryptophan recoveries (n=8) 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 juices and lassi (relative standard deviation in brackets, n=4)

	Protein content K-439 [g/100 ml]	Protein content K-436 [g/100 ml]
Fruit juice	0.82 (0.30 %)	0.84 (0.31 %)
Beetroot juice	0.78 (0.75 %)	0.80 (0.63 %)
India lassi	2.69 (0.58 %)	2.67 (0.27 %)

Conclusion

The determination of protein contents in juice and lassi according to Kjeldahl using SpeedDigester K-436, K-439, and KjelFlex K-360 provides reliable and reproducible results with low relative standard deviations.

References

SLMB 22/4.1 – 4.2
DIN EN ISO 8968-1:2001 appendix A
LFGB §64 L01.00-10/1 appendix A

Operation manual SpeedDigester K-425 / K-436
Operation manual SpeedDigester K-439
Operation manual Kjeldahl Sampler System K-370/K-371

For more detailed information please refer to Application Note 046/2010

1 Introduction

An easy and reliable method for the determination of total nitrogen and protein in juice and lassi according to the Kjeldahl method, as described in the Swiss Food Manual 22/4.1 – 4.2 and other 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 Schott TitroLine easy.

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)
- TitroLine easy (Schott)
- Connecting cable from Titrator to K-360 (Büchi No. 043618)
- 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

- Fruit juice
- Beetroot juice
- India Lassi

The samples were purchased at a local supermarket.
The samples were homogenized by shaking.

5 Procedure

The determination of nitrogen and protein in beverages 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 Schott TitroLine easy

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 “beverages” method (K-439) or the parameters listed in Table 1

5.2 Digestion method - samples

- Place approx. 5 - 8 g of the sample (depends on concentration of nitrogen 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 “beverages” 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 Schott TitroLine easy.

Table 2: Distillation and titration parameters

KjelFlex K-360		Schott TitroLine easy	
Water	80 ml	Titration Solution	H_2SO_4 0.25 mol/l
Sodium Hydroxide	80 ml	Internal Method	pH fast strong
Boric Acid 4 %	50 ml	Endpoint	pH 4.6
Reaction Time	5 s		
Steam Power	100 %		
Distillation Time	240 s		
Titration Start	240 s		
Titration Type	Boric Acid		
Stirrer Speed Dist.	5		
Stirrer Speed Titr.	7		

5.4 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.

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

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

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

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 (general factor for food: 6.25)

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 juices and lassi

The results of the determination of nitrogen in juices and lassi are presented in Tables 4 - 6.

Table 4: Results for the determination of nitrogen in fruit juice with K-439

Fruit juice	m_{Sample} [g]	V_{Sample} [ml]	%N	%P	P [g / 100 ml]
Sample 1	8.1081	3.84	0.126	0.79	0.83
Sample 2	8.1494	3.84	0.125	0.78	0.82
Sample 3	8.1715	3.85	0.125	0.78	0.82
Sample 4	8.1884	3.85	0.125	0.78	0.82
Average	-	-	0.125	0.78	**0.82**
Rsd [%]	-	-	0.30	0.30	**0.30**

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

The experimental protein content [%] was re-calculated taking the density (1.053) into account in order to obtain the protein content as g/100 ml.

Table 5: Results for the determination of nitrogen in beetroot juice with K-439

Beetroot juice	m_{Sample} [g]	V_{Sample} [ml]	%N	%P	P [g / 100 ml]
Sample 1	6.1298	2.79	0.118	0.74	0.78
Sample 2	6.0555	2.79	0.119	0.75	0.78
Sample 3	6.2389	2.83	0.118	0.74	0.77
Sample 4	6.1551	2.83	0.119	0.75	0.78
Average	-	-	0.119	0.74	**0.78**
Rsd [%]	-	-	0.75	0.75	**0.75**

The mean blank volume for this sample was 0.208 ml (n = 4).

The experimental protein content [%] was re-calculated taking the density (1.050) into account in order to obtain the protein content as g/100 ml.

Table 6: Results for the determination of nitrogen in India lassi with K-439

India lassi	m_{Sample} [g]	V_{Sample} [ml]	%N	%P	P [g / 100 ml]
Sample 1	4.8904	7.35	0.409	2.55	2.69
Sample 2	5.1374	7.73	0.410	2.56	2.69
Sample 3	4.9272	7.44	0.411	2.57	2.70
Sample 4	5.1390	7.65	0.405	2.53	2.67
Average	-	-	0.409	0.78	**2.69**
Rsd [%]	-	-	0.58	0.58	**0.58**

The mean blank volume for this sample was 0.215 ml (n = 4).

The experimental protein content [%] was re-calculated taking the density (1.052) into account in order to obtain the protein content as g/100 ml.

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 juices and lassi

The results of the determination of nitrogen in juices and lassi are presented in Tables 8 - 10.

Table 8: Results for the determination of nitrogen in fruit juice with K-436

Fruit juice	m_{Sample} [g]	V_{Sample} [ml]	%N	%P	P [g / 100 ml]
Sample 1	8.2553	3.96	0.127	0.80	0.84
Sample 2	8.0501	3.86	0.127	0.79	0.84
Sample 3	8.1229	3.92	0.128	0.80	0.84
Sample 4	8.1340	3.91	0.127	0.80	0.84
Average	-	-	0.127	0.80	**0.84**
Rsd [%]	-	-	0.31	0.31	**0.31**

The mean blank volume for this sample was 0.210 ml (n = 4).

The experimental protein content [%] was re-calculated taking the density (1.053) into account in order to obtain the protein content as g/100 ml.

Table 9: Results for the determination of nitrogen in beetroot juice with K-436

Beetroot juice	m_{Sample} [g]	V_{Sample} [ml]	%N	%P	P [g / 100 ml]
Sample 1	6.0242	2.82	0.121	0.76	0.80
Sample 2	6.1176	2.85	0.121	0.76	0.79
Sample 3	6.0266	2.80	0.121	0.75	0.79
Sample 4	6.0752	2.86	0.122	0.76	0.80
Average	-	-	0.121	0.76	**0.80**
Rsd [%]	-	-	0.63	0.63	**0.63**

The mean blank volume for this sample was 0.208 ml (n = 4).

The experimental protein content [%] was re-calculated taking the density (1.050) into account in order to obtain the protein content as g/100 ml.

Table 10: Results for the determination of nitrogen in India lassi with K-436

India lassi	m_{Sample} [g]	V_{Sample} [ml]	%N	%P	P [g / 100 ml]
Sample 1	5.0600	7.54	0.406	2.54	2.67
Sample 2	5.0149	7.47	0.406	2.54	2.67
Sample 3	5.0400	7.52	0.406	2.54	2.67
Sample 4	5.1632	7.73	0.408	2.55	2.68
Average	-	-	0.406	2.54	**2.67**
Rsd [%]	-	-	0.27	0.27	**0.27**

The mean blank volume for this sample was 0.210 ml (n = 4).

The experimental protein content [%] was re-calculated taking the density (1.052) into account in order to obtain the protein content as g/100 ml.

7 Comparison to Standard Methods

This application note is based on the standard method SLMB 22/4.1 - 4.2.

Table 9: Differentiation from the standard method

	This application note	Standard method	Notes/Impact
Catalyst	10 g Tablets	15 g Tablets	Higher amount of catalyst can lead to dry samples. The ratio 10 g catalyst / 20 ml acid gives reliable results.
Sodium hydroxide	Conc.: 32 %	Conc.: 50 %	No impact. Sodium hydroxide 32% is more gentle to the pump than higher concentrated alkali.
Boric acid	4 % adjusted to pH 4.65	3 %	No impact.
Titration solution sulfuric acid	Conc.: 0.1 mol/l	Conc.: 0.05 mol/l	No impact. The volume of the titration solution has to be in the optimal range from the titrator.

8 Conclusion

The determination of nitrogen and protein in juices and lassi using the SpeedDigester K-436, K-439, and KjelFlex K-360 provides reliable and reproducible results 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 % [1], [2].

9 References

[1] DIN EN ISO 8968-1:2001 appendix A
[2] LFGB §64 L01.00-10/1 appendix A

SLMB 22/4.1 – 4.2

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 TitroLine easy (Schott)

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