Application Note

085/2012

Digest Automat K-438

KjelMaster K-375 with KjelSampler K-376

Nitrogen and Protein Determination in Animal Feed According to the Kjeldahl Method

Nitrogen and Protein Determination in Animal Feed According to the Kjeldahl Method

The determination of protein in feed is a routine procedure for quality assurance and labelling. A simple and fast method for protein determination in feed products according to the ISO 5983-2:2009 and AOAC 954.01 regulations is introduced below. The sample is digested with sulfuric acid and Kjeldahl Tablets Titanium using the digest Automat K-438, followed by distillation and titration with the KjelMaster K-375 with KjelSampler K-376.

Introduction

Protein determination is one of the key analyses performed in the feed 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 protein factor (6.25 for feed products) to obtain the protein content.

Experimental

Instrumentation:

Digest Automat K-438
KjelMaster K-375 with KjelSampler K-376

Samples:

Dog food with a labelled protein content of 11 g/100 g, rabbit food with a labelled protein content of 15 g/100 g and cat food with 37 g/100 g.

Determination:

The samples were added directly into a sample tube as described in Table 1. A portion of 15 ml sulfuric acid, two titanium tablets and one antifoam tablet were added, and the digestion was performed using the parameters specified in Table 2. After digestion the ammonia of the sample was distilled into a boric acid solution by steam distillation and titrated with sulfuric acid (Table 3).

The method was verified by using 0.15 g tryptophane as a reference substance.

Table 1: Weight for each sample

	Sample weight [g]
Dog food	1.0
Rabbit food	1.0
Cat food	0.5

Table 2: Temperature profile for digestion with the K-438

Step	Temp. [°C]	Time [min]
Preheat	320	-
1	420	120
Cooling	-	30

Table 3: Parameters for distillation and titration using the KjelMaster K-375 with KjelSampler K-376

Method Parameters			
H_2O volume	50 ml	Steam output	100%
NaOH volume	60 ml	Receiving solution	30 ml H_3BO_3 4%
Reaction time	5 s	Titration solution	H_2SO_4 0.1 mol/l
Dist. mode	Fixed time	Endpoint pH	4.65
Dist. time	180 s	Stirrer sp. titr.	7
Stirrer sp. dist.	5	Titr. algorithm	Optimal

Results

The tryptophane recoveries were 100.0 %, rsd = 0.2 % (n=4). The determined protein contents are presented in Table 4.

Table 4: Determined protein contents (rsd in brackets, n=4)

Product	Labelled protein content	Determined protein content
Dog food	11 g/100 g	10.45 g/100 g (0.2%)
Rabbit food	15 g/100 g	14.79 g/100 g (0.5%)
Cat food	37 g/100 g	38.40 g/100 g (0.2%)

Conclusion

The determination of protein contents in feed products using the K-438 and KjelMaster K-375 with KjelSampler K-376 provides reliable and reproducible results that correspond to the labelled values with low relative standard deviations. The total digestion time is approx. 120 min.

References

ISO 5983-2:2009
AOAC 954.01

Operation manual DigestAutomat K-432/ K-438
Operation manual KjelMaster K-375 with KjelSampler K-376

For more detailed information and safety considerations please refer to Application Note 085/2012.

1 Introduction

An easy and reliable method for determining nitrogen and protein contents in feed products according to the Kjeldahl method, as described in the ISO 5983-2:2009, and AOAC 954.01 regulations, is introduced below. The samples are digested using the Digest Automat K-438. The distillation and boric acid titration are performed using the KjelMaster K-375 with KjelSampler K-376.

2 Equipment

- Digest Automat K-438 (the parameters used are also valid for K-437)
- 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

- Sulfuric acid conc 98 %, Fluka (84727)
- Titanium, BUCHI Kjeldahl Tablet (11057980)
- Antifoam, BUCHI Kjeldahl Tablet (11057984)
- Sodium hydroxide 32 %, Brenntag (81980-452)
- Boric acid 4 %, 200 g of boric acid, Brenntag (80948-155) diluted to 5 l with deionized water, pH adjusted to 4.65
- Sulfuric acid 0.1 mol/l, Fluka (35357) standard solution
- Neutralization solution for the Scrubber: 600 g of sodium carbonate, calcined, technical, Synopharm (0179420) about 2 ml of ethanol and a spatula tip of bromthymol blue, Fluka (18460) diluted to 3 l with distilled water
- DL-Tryptophan (Assay >99 %), Alfa Aesar (L05936)
- Sucrose, Riedel-de Haën (16104)

For a safe handling please pay attention to all corresponding MSDS data sheets!

Samples:

- Dog food with a labelled protein content of 11 g/100 g
- Rabbit food with a labelled protein content of 15 g/100 g
- Cat food with a labelled protein content of 37 g/100 g

The samples were purchased at a local supermarket.

The samples were mixed for 10 s up to visual homogeneity.

4 Procedure

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

- Homogenization of the samples
- Digestion of the samples, with the Digest Automat K-438
- Distillation and titration of the samples, using the KjelMaster K-375 with KjelSampler K-376

4.1 Digestion method - tryptophane (verification of the method)

- Preheat the Digest Automat K-438 according to the parameters listed in Table 2
- Place approx. 0.15 g of tryptophane and 0.7 g sucrose into a 300 ml sample tube
- Add two Titanium Tablets, one Antifoam Tablet and 15 ml sulfuric acid (98 %)
- Prepare additional blanks, 2 ml water and 0.7 g of sucrose as a sample
- Carefully suspend the sample by gently swirling the tube
- Connect the Scrubber K-415 to the Digest Automat K-438 for absorbing the acid fumes created during the digestion
- Insert the rack containing the samples into the lift and immediately start the digestion according to the parameters listed in Table 2

4.2 Digestion method - samples

- Preheat the Digest Automat K-438 according to the parameters listed in Table 2
- Place each sample into a 300 ml sample tube as described in Table 1

Table 1: Weight for each sample

Sample	Weight [g]
Dog food	1.0
Rabbit food	1.0
Cat food	0.5

- Add 2 Titanium tablets, one Antifoam Tablet and 15 ml of sulfuric acid (98 %) to each tube
- Prepare additional blanks, 2 ml water and 0.7 g of sucrose as a sample
- Connect the Scrubber K-415 to the K-438 for absorbing the acid fumes created during digestion
- Insert the rack containing 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-438

Step	Temperature [°C]	Time [min]
Preheating	320	-
1	420	120
Cooling	-	30

- If the liquid inside the sample tube is not clear and blue-green, digest for an additional 30 min at 420 °C.

- Let the samples cool down

NOTE: The samples should be clear-green immediately after the digestion. A darkening of the clear liquid samples during the cooling down process is possible but does not affect the results.

4.3 Distillation and titration

Distill the samples according to the parameters listed in Table 3

Table 3: Distillation and titration using the KjelMaster K-375 with KjelSampler 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	Standard
Distillation mode	Fixed time	Measuring mode	Endpoint pH
Distillation time	180 s	Endpoint pH	4.65
Stirrer speed distillation	5	Stirrer speed titration	7
Steam output	100 %	Titration start volume	0 ml
Titration type	Boric acid	Titration algorithm	Optimal
Receiving solution vol.	30 ml		

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.

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

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

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

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 (6.25 for feed products)

5 Results

5.1 Recovery of tryptophane

The results of the nitrogen determination and recovery in tryptophane are presented in Table 4. The nominal value of tryptophane (assay: ≥ 99.0 %) is 13.72 % nitrogen (the value corrected with the assay is 13.58 % nitrogen). The recoveries are within the specification of > 98.5 % [1].

Table 4: Results for the recovery of nitrogen in tryptophane

Tryptophan	m_{Sample} [g]	V_{Sample} [ml]	%N	Recovery [%]
Sample 1	0.1508	7.504	13.578	99.9
Sample 2	0.1538	7.662	13.601	100.1
Sample 3	0.1536	7.618	13.538	99.7
Sample 4	0.1576	7.840	13.589	100.0
Average	**-**	**-**	**13.577**	**100.0**
Rsd [%]	**-**	**-**	**0.2**	**0.2**

The mean blank volume for this sample was 0.195 ml (n = 4).

5.2 Protein determination in feed products

The results of the determination of nitrogen in feed products are presented in Tables 5 - 7.

Table 5: Results for the determination of nitrogen in dog food (declared protein content 11 g/100 g)

Dog food	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	1.0672	6.580	1.676	10.48
Sample 2	1.0460	6.438	1.672	10.45
Sample 3	1.0405	6.388	1.667	10.42
Sample 4	0.9751	6.024	1.675	10.47
Average	**-**	**-**	**1.672**	**10.45**
Rsd [%]	**-**	**-**	**0.2**	**0.2**

The mean blank volume for this sample was 0.195 ml (n = 4).

Table 6: Results for the determination of nitrogen in rabbit food (declared protein content 15 g/100 g)

Rabbit food	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	1.0240	8.772	2.346	14.67
Sample 2	1.0374	8.994	2.376	14.85
Sample 3	0.9979	8.620	2.365	14.78
Sample 4	1.0072	8.744	2.378	14.86
Average	**-**	**-**	**2.366**	**14.79**
Rsd [%]	**-**	**-**	**0.5**	**0.5**

The mean blank volume for this sample was 0.195 ml (n = 4).

Table 7: Results for the determination of nitrogen in cat food (declared protein content 37 g/100 g)

Cat food	m_{Sample} [g]	V_{Sample} [ml]	%N	%P
Sample 1	0.5105	11.365	6.129	38.31
Sample 2	0.4940	11.030	6.144	38.40
Sample 3	0.5212	11.660	6.162	38.51
Sample 4	0.5005	11.164	6.139	38.37
Average	**-**	**-**	**6.144**	**38.40**
Rsd [%]	**-**	**-**	**0.2**	**0.2**

The mean blank volume for this sample was 0.195 ml (n = 4).

6 Comparison to Standard Methods

This application note is based on the standard methods ISO 5983-2:2009 and AOAC 954.01. The standard method AOAC 954.01 does only use 2 g sucrose as blanks.

Table 8: Differentiation from the standard methods

	This application note	Standard methods	Notes/Impact
Catalyst	2 titanium tablets (each 3.5 g K_2SO_4; 0.11 g $CuSO_4$; 0.11 g TiO_2)	ISO: 2 tablets (each 3.5 g K_2SO_4; 0.4 g $CuSO_4$) AOAC: 15 g K_2SO_4; 0.65 g Hg	Easy to handle especially in routine analytics. The choice of catalyst does not influence the result. No toxic Hg.
Sulfuric acid	15 ml	ISO: 12 - 15 ml AOAC: 25 ml	No impact. Same ratio of sulfuric acid / catalyst
Water	50 ml	ISO: 80 ml AOAC: 200 ml	The K-375 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 methods.
Sodium hydroxide	60 ml (Conc.: 32%)	ISO: 50 ml (Conc.: 40 %) AOAC: 80 ml (Conc.: 32 %)	No impact. Same ratio of sodium hydroxide / sulfuric acid. Sodium hydroxide 32% is more gentle to the pump than higher concentrated alkali. The sample solution must be strongly alkaline.
Receiving solution	30 ml boric acid solution 4 %	ISO: 25 - 30 ml boric acid solution 4 % AOAC: 0.5 N H_2SO_4 (Back titration)	No impact. The boric acid titration and the back titration lead to equal results.
Titration solution	H_2SO_4 0.2 N	ISO: HCl 0.1 N AOAC: 0.1 N NaOH (Back titration)	No impact. The volume of titration solution should be between 3 and 17 ml.

7 Conclusion

The determination of nitrogen and protein in feed products using the Digest Automat K-438, KjelMaster K-375, and KjelSampler K-376 provides reliable and reproducible results that correspond to the labelled values with low relative standard deviations. The recoveries with tryptophane were 100.0 % and are within the specification of > 98.5 % [1].

The most important characteristics of the latest version of the fully-automatic Kjeldahl sampler system, KjelMaster K-375 with KjelSampler K-376, are the convenient and intuitive operation, short process time as well as sophisticated data management. In combination with the Digest Automat K-438, personnel attendance during sample analysis is significantly reduced.

8 References

[1] ISO 5983-2:2009

AOAC 954.01

Operation manual DigestAutomat K-432/ K-438

Operation manual of Scrubber B-415

Operation manual KjelMaster K-375 with KjelSampler K-376

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