Application Note No. 113/2013
Nitrogen & protein determination in dry pet food

KjelDigester K-449, KjelMaster K-375 with KjelSampler K-376:
Accelerated Nitrogen and Protein Determination in dry Pet food with Kjeldahl Tablets and Hydrogen Peroxide

1 Introduction

An easy and reliable method for the determination of total nitrogen and protein in dry pet food by the use of hydrogen peroxide, according to ISO 5983-2, is introduced below. The samples are digested using the KjelDigester K-449. The distillation and boric acid titration are performed with the KjelMaster K-375 with KjelSampler K-376. The combination of the accelerated digestion method, using the Kjeldahl Tablet Titanium in combination with hydrogen peroxide, and the KjelMaster system K-375/K-376 increases the sample throughput.

2 Equipment

- KjelDigester K-449 (the parameters used are also valid for K-446)
- Scrubber K-415 TripleScrub ^{ECO}
- KjelMaster K-375 with KjelSampler K-376
- Mixer, Retsch Grindomix GM200
- Analytical balance (accuracy ± 0.1 mg)

Safety accessories**:**

- User protection shield, BUCHI (11057889)
- Hirschmann bottle top dispenser ceramus® 5-30 mL, VWR (613-3243) with ceramus® discharge tube, spiral-shaped, VWR (612-0917)

Figure 1: Safety accessories for the digestion with hydrogen peroxide and Kjeldahl Tablet Titanium

3 Chemicals and Materials

Chemicals:

- Sulfuric acid conc 98 %, Merck (1007482500)
- Titanium, BUCHI Kjeldahl Tablet (11057980)
- Antifoam, BUCHI Kjeldahl Tablet (11057983)
- Hydrogen peroxide, 30 %, Fluka (95302)
- Sodium hydroxide 32 %, Brenntag (81980-452)
- Boric acid 4 %, 400 g boric acid, Brenntag (80948-155) diluted to 10 L with deionized water, pH adjusted to 4.65
- 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
- Acetanilide, assay 99 %, Fluka (112933)

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

Samples:

- Cat food with a labelled protein content of 37 g/100 g
- Rabbit food with a labelled protein content of 15 g/100 g
- Dog food with a labelled protein content of 12 g/100 g

The samples were purchased at a local supermarket.

4 Procedure

The determination of nitrogen and protein in dry pet food includes the following steps:

- Homogenizsation of the samples by grinding
- Digestion of the sample, using K-449 (K-446 respectively)
- Distillation and titration of the sample, using KjelMaster system K-375/K-376

4.1 Digestion method – acetanilide (verification of the method)

1. Start the KjelDigester K-449 according to the parameters listed in Table 1
2. Place 0.15 g acetanilide in a 300 mL sample tube
3. Add 1 Titanium tablet, 1 Antifoam tablet and 12 mL of sulfuric acid (conc. 98 %)
4. Prepare additional blanks, chemicals without sample
5. Connect the Scrubber K-415 to the K-449 for absorbing the acid fumes created during digestion
6. Place the rack under the fume hood and affix the user protection shield
7. *Slowly* add 5 mL hydrogen peroxide (30 %) with the dispenser down the glass wall of the sample tube, wait until the fuming stops and the reaction subsides.
8. Insert the rack containing the user protection shield and the samples into the cooling position and mount the suction module onto the samples, immediately start the digestion according to the parameters listed in Table 1.
9. Let the samples cool down when the digestion is completed.

4.2 Digestion method – samples

1. Start the KjelDigester K-449 according to the parameters listed in Table 1

Table 1: Temperature profile for digestion with the K-449

Step	Temperature [°C]	Time [min]
1	300	0
2	420	60
Cooling	–	25

2. Place each sample in a 300 mL sample tube as described in Table 2
3. Add 1 Titanium tablet, 1 Antifoam tablet and 12 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. Place the rack under the fume hood and affix the user protection shield
7. *Slowly* add 5 mL hydrogen peroxide (30 %) with the dispenser down the glass wall of the sample tube, wait until the fuming stops and the reaction subsides.
8. Insert the rack containing the user protection shield and the samples into the cooling position and mount the suction module onto the samples, immediately start the digestion according to the parameters listed in Table 1.

Table 2: Weight of each sample [1]

Expected protein content [%]	Weight [g]
3 – 30	1.0
30 – 80	0.5
> 80	0.3

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

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.

9. Let the samples cool down when the digestion is completed.

4.3 Distillation and titration

Distill the samples according to the parameters listed in Table 2.

Table 2: 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	50 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.	50 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. For the reference substance, the purity of the acetanilide is considered in equation (4).

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

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

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

$$\%N_{Ac} = \frac{\%N \bullet 100}{P} \quad (4)$$

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

$\%N_{Ac}$: percentage of weight of nitrogen corrected for the purity of reference substance acetanilide [%]

%P : percentage of weight of protein

P : purity of the reference substance acetanilide [%]

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

5 Results

5.1. Recovery of acetanilide

The results of nitrogen determination and recovery for acetanilide analysis (assay > 99 %) are presented in Table 4. The nominal value of tryptophan is 10.36 % nitrogen. The recoveries are within the specification of > 99.5 % [1]

Table 4: Results of the recovery of nitrogen in acetanilide

Acetanilide	m_{Sample} [g]	V_{Sample} [mL]	$\%N_{Ac}$	Recovery [%]
Sample 1	0.1534	5.838	10.40	100.4
Sample 2	0.1448	5.513	10.39	100.2
Sample 3	0.1546	5.862	10.37	100.0
Sample 4	0.1651	6.279	10.42	100.6
Average [%]	–	–	10.39	100.3
Rsd [%]	–	–	0.2	0.2

The mean blank volume (V_{Blank}) was 0.199 mL (n = 4).

5.2 Protein determination in dry pet food

The results of the determination of nitrogen and protein contents in different pet food are presented in Tables 5 – 7.

Table 5: Results of the determination of nitrogen and protein in cat food (protein content 37 g/100 g)

Cat food	m_{Sample} [g]	V_{Sample} [mL]	%N	%P
Sample 1	0.4997	11.186	6.160	38.5
Sample 2	0.6254	13.889	6.133	38.3
Sample 3	0.4859	10.650	6.140	38.4
Sample 4	0.5334	11.909	6.150	38.4
Average [%]	–	–	6.146	38.4
Rsd [%]	–	–	0.2	0.2

The mean blank volume (V_{Blank}) was 0.199 mL (n = 4).

Table 6: Results of the determination of nitrogen and protein in dog food (protein content 12 g/100 g)

Dog food	m_{Sample} [g]	V_{sample} [mL]	%N	%P
Sample 1	1.0103	7.707	2.082	13.0
Sample 2	0.9792	7.462	2.078	13.0
Sample 3	1.0403	7.909	2.076	13.0
Sample 4	1.0237	7.828	2.088	13.1
Average [%]	–	–	2.081	13.0
Rsd [%]	–	–	0.3	0.3

The mean blank volume (V_{blank}) was 0.199 mL (n = 4).

Table 7: Results of the determination of nitrogen and protein in rabbit food (protein content 15 g/100 g)

Rabbit food	m_{Sample} [g]	V_{sample} [mL]	%N	%P
Sample 1	0.9966	8.755	2.405	15.0
Sample 2	0.9918	8.709	2.404	15.0
Sample 3	0.9619	8.419	2.394	15.0
Sample 4	1.0150	8.908	2.404	15.0
Average [%]	–	–	2.402	15.0
Rsd [%]	–	–	0.2	0.2

The mean blank volume (V_{Blank}) was 0.199 mL (n = 4).

6 Comparison to official regulations

This application note is based on the standard method ISO 5983-2 and AOAC 976.05 with minor differences. These differences are shown in Table 8.

Table 8: Differences to ISO 8968-3 and AOAC 976.05

	Application note	ISO 5983-3	AOAC 976.05	Notes/Impact
Catalyst	1 × 3.7 g Tablets Composition 94.4 % K_2SO_4 2.8 % TiO_2 2.8 % $CuSO_4*5H_2O$	2 × 3.9 g Tablets Composition 89.7 % K_2SO_4 10.3 % $CuSO_4*5H_2O$	3x 5.25gTablets Composition 95.2 % K_2SO_4 4.8 % HgO	Just one tablet to lower the risk of crystallization. No use of toxic HgO
Anitfoam agent	Antifoam tablet containing silicone	Aqueous emulsion containing silicone	-	No impact, same antifoam agent.
Hydrogen peroxide	5 mL (Conc. 30-35%)	5 mL (Conc. 30-35%)	10 mL (Conc. 30-35%)	No impact.
Boric acid	50 mL	25 - 30 mL	50 mL mixed indicator solution	In the conducted experiments a higher amount of boric acid was used then described in standard methods as the tip of the condenser outlet and the electrode have to be immersed in the solution.
Sodium hydroxide	Conc. 32 %	Conc. 40 %	Conc 40 % containing thiosulfate solution	No impact, more gentle to the NaOH pump.No thiosulfate necessary, no use of mercury.
Titration solution	H_2SO_4 0.2 N	HCl 0.1 N	H_2SO_4 0.6 %	No impact, consumption of the titration solution shoud be between 3 - 17 mL

7 Conclusion

The determination of nitrogen and protein in dry fet food using the KjelDigester K-449 and Kjeldahl sampler system K-375/K-376 provides reliable and reproducible results. These results correspond well to the labelled values of the different pet food with low relative standard deviations (rsd) and a short digestion time of 60 min. The recovery with acetanilide was 100.3 % (rsd = 0.82 %), which was within the specification of > 99.5 % [1].
In combination with the accelerated digestion method using the KjelDigester K-449, Kjeldahl Tablet Titanium and hydrogen peroxide, the time needed for sample analysis is significantly reduced and therefore throughput increased.
With the KjelDigester K-449 the digestion process (including preheating, digestion and cooling) is very fast and fully automated. Together with the fully-automatic KjelMaster system K-375/K-376, the time to result is significantly reduced and it offers fully walk-away convenience.

8 References

[1] ISO 5983-2 Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein

AOAC 976.05 Protein (Crude) in Animal Feed and Pet Food

KjelCalc App

Operation Manual of KjelDigester K-446/K-449
Operation Manual of Scrubber K-415
Operation Manual of KjelMaster system K-375/K376