Short Note No. 186/2015
Nitrogen & protein determination in feed

KjelDigester K-449, KjelMaster K-375 with KjelSampler K-376:
Accelerated Nitrogen and Protein Determination in Feed According to the Kjeldahl Method by Digestion with Kjeldahl Tablets and Hydrogen Peroxide Followed by Colorimetric Titration

A reliable and efficient method for the determination of total nitrogen and protein in feed, according to ISO 5983-2 [1] and AOAC 2001.11 [2], is presented. The samples are digested using Kjeldahl Tablets, hydrogen peroxide and the KjelDigester K-449. Distillation and boric acid titration are performed with the KjelMaster System K-375 / K-376 equipped with a colorimetric sensor.

1. Introduction

Protein determination is one of the key analyses performed in the food and feed industry. The samples require digestion with sulfuric acid and hydrogen peroxide to convert nitrogen to ammonium sulphate within 60 minutes (rabbit feed, tryptophan) or 75 minutes (reference material). After conversion to ammonia through alkalinization with sodium hydroxide, the sample is steam distilled into a boric acid receiver, followed by a colorimetric titration with sulfuric acid solution. The measured nitrogen content is multiplied by a sample-specific protein factor (6.38 for dairy feed, 6.25 for other feed) to obtain the protein content.

2. Experimental

Equipment: KjelDigester K-449 with protection shield
KjelMaster K-375 with KjelSampler K-376

Samples: Certified reference material - BCR® - 708 – dairy feed with a declared protein content of 24.0 g/100 g (uncertainity 1.2 g/100 g), rabbit feed with a declared protein content of 12.5 g/100 g, tryptophan

Determination: The samples were homogenized with a mixer and placed into a sample tube as described in Table 1. One Titanium tablets, 12 mL of sulfuric acid (conc. 98 %) were added.

Table 1: Sample weight

Samples	Sample weight [g]
Tryptophan	0.15
Certified reference material – dairy feed	0.7
Rabbit feed	1

Before starting the digestion, the rack was placed under the fume hood and the user protection shield must be affixed. 6 mL hydrogen peroxide (30 %) were added slowly down the glass wall of the sample tubes. When the fuming stopped, the digestion was initiated.

The digestion was performed using the K-449, applying the temperature profile specified in Table 2.

Table 2: Temperature profile for digestion with the K-449

Step	Temperature [°C]	Time [min]
1	340	0
2	420	60 (rabbit feed) 75 (reference material)
Cooling	---	30

After digestion the ammonia of the sample was distilled into a boric acid solution by steam distillation and titrated with sulfuric acid performed with the KjelMaster system K-375 / K-376 by colorimetric titration using the Sher indicator. All details about the used parameters, as for example the volume of NaOH, distillation time and titrant concentration, are described in the according application note [3].
For colorimetric titration it`s necessary to determine the setpoint of the boric acid solution in advance to the blank and sample determination. This procedure including specific preparation of the sensor is described in the Technical Note 179/2015 [4] “Colorimetric titration procedure using Sher indicator”.

3. Results

The recoveries of the tryptophan reference were 100.0 %, rsd = 0.37 % (n=3). The determined protein contents of the samples are presented in Table 3.

Table 3: Determined protein contents (rsd in brackets, n=5)

Product	Protein content [g/100 g]
Certified reference material – dairy feed	23.6 (0.5)
Rabbit feed	12.2 (0.7)

4. Conclusion

The determination of nitrogen and protein in feed using the KjelDigester K-449 and KjelMaster system K-375 / K-376 by colorimetric titration provides reliable and reproducible results. The here found results correspond well to the labelled values of the rabbit and certified reference dairy feed with low relative standard deviations (rsd).
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 from 120 min [5] to 60 min or 75 min and therefore the throughput increased.
The combination with the fully-automatic KjelMaster system K-375 / K-376, allows unattended operation and highest sample throughput of about 120 samples per 9 hours working day.

5. References

[1] ISO 5983-2 Animal feeding stuffs – Determination of nitrogen content and calculation of crude protein content, Part 2: Block digestion and steam distillation method
[2] AOAC 2001.11: Protein (Crude) in Animal Feed, Forage (Plant Tissue), Grain, and Oilseeds
[3] Application Note 186/2015 Nitrogen and protein determination in feed
[4] Technical Note 179/2015 Colorimetric titration procedure using Sher indicator
[5] Application Note 085/2012 Nitrogen and Protein Determination in Animal Feed According to the Kjeldahl Method

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