Short Note No 197/2015
Nitrogen and protein determination in dairy products

KjelDigester K-449, KjelMaster K-375 with KjelSampler K-376:
Accelerated Nitrogen and Protein Determination in Dairy Products According to the Kjeldahl Method by Digestion with Kjeldahl Tablets and Hydrogen Peroxide Followed by Colorimetric 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 dairy products, according to ISO 8968-3 and AOAC 991.20 is introduced below [1,2]. The sample is digested with sulfuric acid, the Kjeldahl Tablet Titanium and hydrogen peroxide using the KjelDigester K-449 – followed by distillation and titration with the KjelMaster system K-375 / K-376. The determined protein contents correspond to the labelled values.

1. 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. Here, digestion was accelerated using hydrogen peroxide. 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 colorimetric titration with a sulfuric acid solution. The nitrogen content is multiplied by a sample-specific protein factor (6.38 for dairy products) to obtain the protein content.

2. Experimental

Equipment: KjelDigester K-449 / KjelMaster K-375 with KjelSampler K-376

Determination: The samples were homogenized and added directly into a sample tube as described in Table 1. One Titanium tablet and 10 mL of sulfuric acid (conc. 98 %) were added.

Table 1: Weight and protein content for each sample

Sample	Labelled Protein content	Sample weight [g]
Skimmed milk	3.4 g / 100 mL	2.0
Cream	2.2 g / 100 g	2.0
Shake milk drink	1.5 g / 100 mL	2.5
Parmesan cheese	33 g / 100 g	0.25

Before starting the digestion, the rack was placed under the fume hood and the user protection affixed. 8 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 and applying the parameters specified in Table 2. The method was verified by measuring 0.12 g glutamic acid as the reference substance.

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

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

After digestion the ammonia of the sample was distilled into a boric acid solution by steam distillation and titrated with sulfuric acid (Table 3) performed by the KjelMaster system K-375 / K-376.

Table 3: Method parameters for distillation and titration with the KjelMaster system K-375 / K-376

H_2O volume	50 mL	Receiving solution	60 mL H_3BO_3 4 %
NaOH volume	45 mL	Titration solution	H_2SO_4 0.05 mol/L
Reaction time	5 s	Sensor type	colorimetric
Dist. mode	fixed time	Titration mode	online
Dist. time	180 s	Titration start time	90 s
Stirrer sp. dist	5	Stirrer sp. titr.	10
Steam output	100 %	Titr. algorithm	optimal

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 for the sensor is described in the Technical Note 179/2015 [3] Colorimetric titration procedure using Sher indicator.

3. Results

The glutamic acid recoveries were 100.3 %, rsd = 0.76 % (n=4). The determined protein contents of the dairy samples are presented in Table 4.

Table 4: Determined protein contents (rsd in brackets, n=3)

Product	Protein content [g/100 g]	Protein content [g/100 mL]
Skimmed milk	3.60 (0.73 %)	3.49 (0.73 %)
Whipping cream	2.06 (0.51 %)	-
Shake milk drink	1.74 (0.28 %)	1.68 (0.28 %)
Parmesan cheese	32.47 (1.54 %)	-

4. Conclusion

The determination of nitrogen and protein in dairy products using the KjelDigester K-449 and KjelMaster system K-375 / K-376 by colorimetric titration provides reliable and well reproducible results. By applying accelerated digestion using hydrogen peroxide, the process time was significantly reduced and therefore the throughput increased.
All mearsured results correspond well to the labelled values of the dairy products. 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] EN ISO 8968-3:2007 Milk and milk products – Determination of nitrogen content - Part 3: Block-digestion method (Semi-micro rapid routine method)
[2] AOAC 991.20 Nitrogen (Total) in Milk – Kjeldahl Method - Block Digestor / Steam Distillation Method

[3] Technical Note 179/2015 Colorimetric titration procedure using Sher indicator

For more detailed information and safety considerations please refer to the Application Note no. 197/2015.