Application Note – N°. 863/2024

Casein determination in milk using micro-Kjeldahl

Abstract: An easy and reliable method for the determination of the casein content in milk is introduced. The sample is digested with the SpeedDigester K-439 using 100 mL micro-Kjeldahl tubes. The distillation is performed with MultiKjel. The obtained results correspond well to literature values. The use of micro-Kjeldahl provides an economic and ecologic advantage. A video is also available on YouTube.

1. Introduction

An easy and reliable method for the determination of the casein content in milk is introduced. Hereby, the Total Kjeldahl Nitrogen (TKN) and the non-casein nitrogen (NCN) content are determined in two different methods, and the casein content calculated by subtraction. In Application Note 861/2024, the TKN determination of milk using micro-Kjeldahl is explained in detail. This application note focuses on the determination of the NCN content and the calculation of the casein content by using TKN values from the Application Note 861/2024.

The samples are digested using the SpeedDigester K-439 with 100 mL micro-Kjeldahl tubes. The steam distillation and titration in boric acid are performed with the MultiKjel in combination with the Metrohm Eco Titrator. This application note complies with official methods e.g. AOAC 998.05 and 17997-1:2004. [1,2]

Micro-Kjeldahl minimizes the amount of sulfuric acid and catalyst for the digestion, and the amount of sodium hydroxide for the distillation step as well. The savings in chemicals provides an economic and ecologic advantage to the classic Kjeldahl method while maintaining its high accuracy and preciseness.

In this application note, bovine drink / skim milk and bovine whole milk were investigated. The distinction between casein and whey proteins can be of great importance e.g. in the making of ice cream UHT products, cheese and yogurt. [3]

2. Equipment

- SpeedDigester K-439 (1154392000) with standard suction module (11055849).
- Sample tubes micro (11057442).
- Rack for 300 mL/micro tubes (11055248).
- Insulation plate for micro tubes (11055204).
- Scrubber K-415 TripleScrub ECO (114152331).
- MultiKjel with Eco Titrator (potentiometric) and recirculating chiller F-314 (11K36531211).
- Volumetric pipette (25 mL).
- Micropipette (1 mL).
- Volumetric flasks (100 mL).
- Funnel and filter paper.
- Beakers or Erlenmeyer flask (e.g. 100 mL).
- Analytical balance (accuracy ± 0.1 mg).

3. Chemicals and Materials

- Sulfuric acid 0.05 mol/L, volumetric solution, titer = 1.0000 Roth (K026.1).
- Sodium hydroxide 32%, Brenntag (81980-452).
- Sulfuric acid 96% VWR (85546.320).
- BUCHI Titanium Micro Kjeldahl tablets (11072628).
- BUCHI ready to use 4% boric acid pH 4.65 with Sher indicator (11064973).
- BUCHI nitrogen-free weighing boats (11060522).
- Sodium acetate ≥ 98%, GPR RECTAPUR® (VWR, Order No. 27650.292).
- Acetic acid ≥ 98%, TECHNICAL (VWR, Order No. 84528.290).
- Stearic acid.

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

4. Procedure

A video of this application is also available on YouTube. This application note focuses on the determination of the NCN content and the calculation of the casein content by using TKN values from the Application Note 861/2024. The TKN determination in milk samples is described in the Application Note 861/2024. The determination of the NCN include the following steps:

- Homogenization of the sample.
- Precipitation and filtration of casein.
- Digestion of the sample, using SpeedDigester K-439.
- A distillation followed by a titration employing MultiKjel coupled with Metrohm Eco Titrator and BasicKjel or EasyKjel with a separate titrator / manual titration.

4.1 Digestion method – tryptophan (verification of the method)

1. Samples were mixed using a spatula.

4.2 Digestion method – samples

2. Place approximately 20 g sample in a 100 mL volumetric flask. Note the weight to the nearest 0.1 mg.
3. Add approximately 75 mL 38–40 °C deionized water.
4. Add 1 mL 10 vol% acetic acid using a micropipette.
5. Stopper the flask and swirl the volumetric flask.
6. Place the flask in a water bath or drying oven at 38 to 40 °C for 10 minutes.
7. Add 1 mL 1 M sodium acetate solution using a micropipette. Swirl to mix.
8. Cool the flask to ambient temperature, ideally 20 °C.
9. Dilute to the 100 mL mark using deionized water.
10. Filter the suspension using funnel and filter paper. Collect the filtrate, which should be clear and free of particles.

4.3 Digestion method

Standard 100 mL micro-Kjeldahl Digestion with Kjeldahl tablets Titanium micro as a catalyst:

11. Place 1 Titanium micro tablet in each tube.
12. Place 25 mL of filtrate in a micro sample tube.
13. Add 6.5 mL of sulfuric acid (conc. 96%) to each tube.
14. Prepare additional blanks, chemicals without sample.
15. Add a digestion rod (043087) and a spatula tip of stearic acid to each tube.
16. Connect the Scrubber K-415 to the SpeedDigester K-439 for absorbing the acid fumes created during digestion.
17. Mount the standard suction module onto the sample tubes.
18. Install the insulation plate for micro tubes on the SpeedDigester K-439.
19. Start the preheating (according to Table 1).
20. Insert the rack with the samples into the cooling position and start preheating step. Once preheating is completed, shift the sample rack in the digestion position and immediately start the digestion according to the parameters listed in Table 1.

Table 1: Temperature ramp for standard digestion with the K-439.

Step	Temperature [°C]	Time [min]
Preheating	350	0
1	350	50
2	490	45
Cooling	-	30

NOTE: As a first indication of successful digestion, the digested sample should be clear and blue green, with acceptable recoveries of a reference substance. Depending on the sample, precipitation or crystallization was observed with no effect on the results.

Let the samples cool down in the cooling position when the digestion is completed.

4.4 Distillation and titration

Distill and titrate according to the parameters listed below. The preparation of the MultiKjel / BasicKjel system could be done by just using the tab “PREP” that combines both Preheating and Priming steps. By activating the AutoDist mode, further preheating or priming is not required even with intermittent breaks in between the determinations.

Table 4: Differences to ISO 17997-1 and AOAC 998.05.

Method parameters MultiKjel		Instrument settings	
Reaction detection	Off	MaxAccuracy mode	On
H_2O volume	26 mL	Chiller / Tap water	Chiller F-314
NaOH volume	30 mL	Chiller temperature	10 °C
Reaction time	5 s	AutoDist mode	On
Steam steps	Fixed time		
Steam power	100%	Automated titration on Eco Titrator	
Level detection	Off	Eco titrator method	
Distillation time	180 s	Titrant	H_2SO_4 0.1 N
Stirrer speed distillation	5	Sensor type	Potentiometric
Titration type	Boric acid titration	Method	Nitrogen (N)
H_3BO_3 volume	60 mL (2%)	Endpoint	pH = 4.65
Stirrer speed titration	8		
Titration start time	180 s		
Sample tube aspiration	20 s		
Reciever aspiration	20 s		

4.5 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. To determine the casein content, the non-casein content, as determined in this application, is deducted from the Total Kjeldahl Nitrogen (TKN), determined in e.g. application note 861/2024. The following equations (1), (2), (3) and (4) are used to calculate the results.

$$w_N = \frac{(V_{Sample} - V_{Blank}) \cdot z \cdot c \cdot f \cdot MN}{m_{Sample} \cdot 4 \cdot 1000} \quad (1)$$

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

$$\%P_{non\text{-}casein} = w_N \cdot PF \cdot 100\% \quad (3)$$

$$\%P_{casein} = \%P_{TKN} - \%P_{non\text{-}casein} \quad (4)$$

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: Titer value (for commercial solutions normally 1.000; refer to the product certificate).
M_N: Molecular weight of nitrogen (14.007 g/mol).
m_{Sample}: Sample weight [g].
1000: Conversion factor [mL to L].
4: Diluting factor.

%N: Percentage weight of nitrogen.
%P: Percentage weight of protein.
PF: Sample-specific protein factor (6.38 for milk products).

5. Result

The results of the determination of casein contents in milk products are presented in Table 3.

Table 3: Results of the determination of casein in milk products using 100 mL micro-Kjeldahl tubes.

Sample	m_{Sample} [g]	V_{Sample} [mL]	%N	$\%P_{casein}$	$\%P_{TKN}$	Mean values	Literature value
Bovine drink milk	20.2603	2.756	0.06%	2.98%	3.35%	**2.98%** rsd : 0.02%	2.95 g/100 mL [5]
	20.1608	2.751	0.06%	2.98%			
Bovine whole milk	20.5006	2.793	0.06%	2.90%	3.27%	**2.90%** rsd : 0.06%	
	20.0588	2.732	0.06%	2.90%			

The mean blank volume (V_{Blank}) was 0.648 mL (n = 2, RSD = 0.55%).

The $\%P_{TKN}$ values are from the Application Note 861/2024.

6. Comparison to Standard Methods

A comparison of this Application Note with the standard methods is shown in Table 4.

Table 4: Differences to ISO 17997-1 and AOAC 998.05.

	Application note	ISO 17997-1	AOAC 998.05	Notes / Impact
Sample amount	20 g	10 g	10 ± 0.1 mL	Double sample amount, no impact on results.
Extract amount	25 mL	50 mL	50 mL	Half the extract amount to negate the double sample amount.
Acetic acid (10 vol%) and sodium acetate (1 M) amount	1 mL each	5.5 mL each	1 mL each	No impact. The ratio must be 1:1.
Catalyst	1 × 1.59 g Tablet Composition 94.4% K_2SO_4 2.8% TiO_2 2.8% $CuSO_4*5H_2O$	15.00 g K_2SO_4, 0.05 g $CuSO_4*5H_2O$	15.00 g K_2SO_4, 0.05 g $CuSO_4*5H_2O$	Less toxicity, for digestion no impact. Less chemicals required.
Sulfuric acid	6.5 mL	25 mL	25 mL	No impact, less chemicals required.
Sodium hydroxide	30 mL (Conc. 32%)	75 mL (50%)	75 mL (50%)	No impact, ensure alkalization, less chemicals required.
Titrant	H_2SO_4 0.1 N	0.1 N HCl	0.1 N HCl	No impact, consumption of the titrant should be 3–17 mL.
Boric acid	60 mL (4%)	50 mL (4%)	50 mL (4%)	No impact, less chemicals required. Alternatively, a back titration is possible to completely omit the use of boric acid.

7. Conclusion

The determination of NCN and casein in milk products with Micro-Kjeldahl using the SpeedDigester K-439 and Kjel Line systems provides reliable and reproducible results, with low relative standard deviations. The determined casein content corresponds well to literature values.

Micro-Kjeldahl minimizes the amount of sulfuric acid and catalyst for the digestion, and the amount of sodium hydroxide for the distillation step as well. The savings in chemicals provides an economic and ecologic advantage to the classic Kjeldahl method while maintaining its high accuracy and preciseness.

With the MaxAccuracy mode and AutoDist mode, samples could be analyzed with or without intermittent breaks between the determinations. These features offer flexibility to an operator without compromising on accuracy and precision of the determination. By coupling the Eco -Titrator to the distillation unit, MultiKjel systems offer easy automation without any manual handling after distillation and titration. Distillation with BasicKjel / EasyKjel followed by separate titration provides a less automated, but more cost-effective alternative with equally quantitative results.

8. References

[1] AOAC 998.05 Noncasein Nitrogen Content of Milk.
[2] ISO 17997–1:2004 Milk – Determination of casein-nitrogen content.
[3] Quantitative Determination of Total Protein, Casein, and Whey Protein of Processed Dairy Products, F. W. Douglas et al., J. Dairy Sci, 65:339–345, 1982.
[4] Advanced Dairy Chemistry Volume 1: Proteins. Ed. P. F. Fox, Elsevier Science Publishers Ltd, Essex, United Kingdom, 1992.
[5] Health-Related Aspects of Milk Proteins, S. H. Davoodi et. al., Iran J Pharm Res. 15(3): 573–591, 2016.

Operation Manual of KjelDigester K-446 / K-449
Operation Manual of Kjel Line K-365