
N/Protein Determination in Milk according to the Kjeldahl method

Reference: **AOAC 991.20** Nitrogen (Total) in Milk; **IDF 20, ISO 8968** Milk - Determination of nitrogen content

Tested with **VELP Scientifica DKL 20 Automatic Kjeldahl Digestion Unit** (Code S30100210) and
UDK 159 Automatic Kjeldahl Distillation&Titration System (Code F30200150).



Introduction

Milk and dairy products play a role of primary importance in the human food and are essential to the balance and the adequacy of the food ration.

The milk composition (water, fat, minerals, lactose and proteins) is an important parameter for its quality evaluation.

The protein fraction includes caseins (80%) and whey proteins (20% such as lactalbumin, lactoglobulin) and it is of interest, not only from the nutritional point of view, but also from a technological one.

In fact, milk proteins have particular chemical properties which contribute to create typical sensory features of dairy products (i.e. cheese and yogurt), influencing the whole food processing.

Protein Determination in Milk according to the Kjeldahl method

Kjeldahl is nowadays the most used method for determining nitrogen and protein contents in foods and feeds, thanks to the high level of precision and reproducibility and to its simple application.

The modern Kjeldahl method consists in a procedure of catalytically supported mineralization of organic material in a boiling mixture of sulfuric acid and sulfate salt at digestion temperatures higher than 400 °C. During the process the organically bonded nitrogen is converted into ammonium sulfate. Alkalizing the digested solution liberates ammonia which is quantitatively steam distilled and determined by titration.

Sample

Liquid bovine semi-skimmed milk

Protein labeled value: 3.25 g/100 ml

Sample Digestion

Stir the milk into a beaker using a VELP magnetic stirrer for 60 sec. at 700 rpm.

Put 5 ml of sample into a 250 ml test tube, by using a pipette.

For each sample, add in the test tube:

- 2 catalyst tablet VCM (code A00000274; 3.5 g K₂SO₄, 0.1 g CuSO₄, 5H₂O Missouri)
- 20 ml concentrated sulphuric acid (96-98%)
- 5 ml of hydrogen peroxide (~ 30%)

Prepare some blanks with all chemicals and without sample.

Connect the Digestion Unit to a proper Aspiration Pump (JP code F30620198) and a Fume Neutralization System (SMS Scrubber code F307C0199) to neutralize the acid fumes created during digestion phase.

Digest the samples for 15 minutes at 150 °C, plus 15 minutes at 250 °C and 40 minutes at 420 °C according to the method "milk and derived products" (n° 1 on DKL 20).

Distillation and Titration

Let the test tubes cool down to 50-60 °C.

Condition the UDK 159 unit by performing the Automatic Check up in Menu-System and a Wash down.

Distill the samples according to the following parameters (pre-defined method n°1):

- H₂O (dilution water): 50 ml
- NaOH (32 %): 70 ml
- H₃BO₃ (4 % with indicators): 30 ml
- H₂SO₄ (0.1 N) as titrant solution
- Protein factor: 6.38

Distillation & Titration analysis time: from 4 minutes for one test.

Typical Results on Semi-Skimmed Milk

The results are automatically calculated by UDK 159 as a percentage of nitrogen and percentage of proteins. This is "protein" on a total nitrogen basis.

Sample quantity (ml)	Nitrogen %	Protein %
5	0.533	3.399
5	0.534	3.409
5	0.537	3.427
5	0.541	3.449
5	0.533	3.398
5	0.536	3.419
5	0.533	3.401
5	0.538	3.431
5	0.535	3.410
5	0.537	3.423
Average ± SD%	0.536 ± 0.003	3.417 ± 0.016
RSD% *	0.490	0.479

Protein Labeled Value: 3.25 g/100 ml

Protein Factor: 6.38

* RSD% = (Standard Deviation * 100) / Average

The complete procedure was verified by using 5 ml of glycine standard solution (3%) containing 28 mg of nitrogen, as reference substance.

The obtained recovery falls into the expected range: between 98% and 102%.

Conclusion

The obtained results are reliable and reproducible in accordance with the expected values, with a low relative standard deviation (RSD < 1%), that means high repeatability of the results.

Benefits of Kjeldahl method by using DKL 20 and UDK 159 are:

- High level of precision and reproducibility
- High productivity
- Worldwide official method
- Reliable and easy method
- Time saving
- Affordable equipment cost
- Moderate running costs