Casein Determination in Milk
according to the Kjeldahl method

Reference: **AOAC 998.05** Non-casein Nitrogen Content of Milk; **AOAC 991.20** Nitrogen (Total) in Milk; **ISO 8968 - 4 FIL 20 - 4 : 2001** Non-protein 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

The milk proteins are the oldest and most widely consumed food proteins. There is currently a large interest in these substances, both in nutritional field and in technological application. *Casein proteins* are a family of proteins involved in the production of cheese and fermented milk. They are very important nitrogen compounds not only for the dairy products production, but they are also additives in medicine, and they have a technical use in cosmetics, paints and adhesives. Casein content is determined by the difference between the *total nitrogen (N_{tot})* content and the *non-casein nitrogen (NCN)*, obtained by the milk, following the procedure indicated in this document.

Casein 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 sulphuric acid and sulphate salt at digestion temperatures higher than 400 °C. During the process the organically bonded nitrogen is converted into ammonium sulphate. Alkalizing the digested solution liberates ammonia which is quantitatively steam distilled and determined by titration.

Sample

Liquid bovine high quality milk, whole and pasteurized

Protein labeled value: 3.35 g/100 ml
Casein content from literature: 2.66%

Sample preparation

The determination of the *non-casein nitrogen (NCN)* is necessary to calculate the casein content in the milk.
The *NCN* is obtained separating and filtrating the milk.

Chemicals and Materials for separating and filtrating

Acetic acid solution 10% - 10 ml acetic acid diluted to 100 ml with deionized water
Sodium acetate solution 1M - 8,2 g sodium acetate diluted to 100 ml with deionized water
Filter paper nitrogen free, high speed filtration

Procedure

The determination of NCN in milk includes the following steps:
- Stir the milk into a beaker using a VELP magnetic stirrer for 60 sec. at 700 rpm.
- Precipitation of the casein and filtration
- Digestion of the filtrate using DKL 20
- Distillation and titration of the sample using UDK 159
- Calculation (see the following formulas)

Place 20 ml of milk, previously thermostated at 20 °C, in a 50 ml volumetric flask with 20 ml of deionized water. Then, put the flask at 37 °C in an Open Circulating Bath (OCB, Code F40300240) for 30 minutes. After this period, add 2 ml of acetic acid solution (10%), swirl to mix and let stand for approximately 10 minutes. Add 2 ml of sodium acetate solution 1M, let the mixture cool down to 20 °C and fill up with deionized water to the calibration mark. Then, filter through a filter paper and collect the entire filtrate.

Sample Digestion

Put 20 ml of filtrate into a 250 ml test tube (Code A00000144), by using a pipette.
In each the test tube add:
- 2 catalyst tablets VCM (code A00000274; 3.5 g K_2SO_4, 0.1 g $CuSO_4$ $5H_2O$ Missouri)
- 4 antifoam tablets VS (Code A00000283)
- 15 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, setting the following ramps in "Customizable Methods": for 15 minutes at 150 °C plus 45 minutes at 200 °C plus 15 minutes at 300 °C plus 60 minutes at 420 °C

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 selecting the predefined method n° 1 :

- H_2O (dilution water): 50 ml
- H_2SO_4 (0.1 N) as titrant solution
- NaOH (32%): 70 ml
- Protein factor: 6.38
- H_3BO_3 (4% with indicators): 30 ml

In UDK 159 settings, set as unit of measure mgN and %N for the final result and as sample quantity "ml".

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

Typical Results

The results of the *non-casein nitrogen (NCN)* are calculated as percentage of nitrogen, using as sample quantity the volume of filtrate (*V filtrate*) multiplied for 0,4. They are based on the following formula:

$$NCN\% = mg\ N\ /\ [(G\ milk/V\ sol)\ x\ 1000\ x\ V\ filtrate]\ x\ 100$$

G milk = milk weight (20 g)
V sol = milk solution containing all the chemicals necessary for separating, filled up to volume (50 ml)
V filtrate= filtrate used to perform 1 analysis (ml)

The obtained results have been exported and multiplied for the whole milk correction factor 0.994 (for semi skimmed milk, the correction factor is 0.998). Casein is calculated taking into account the measured total nitrogen content (N_{tot}) of 0.525% in whole milk. (For N_{tot}, see Application Note "*N/Protein Determination in Milk according to the Kjeldahl method*")

$$Casein\% = 6.38\ x\ (\%N_{tot} - \%NCN)$$

Filtrate quantity (ml)	NCN %*	Casein %
20.000	0.108	2.658
20.000	0.109	2.653
20.000	0.110	2.646
Average ± SD%	**0.109 ± 0.001**	**2.652 ± 0.006**
RSD% **	**0.917**	**0.243**

* already corrected with 0,994 factor   ** RSD% = (Standard Deviation x 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