

N/Protein Determination in Probiotic Drink according to the Kjeldahl method

Reference: IDF 20-1, ISO 8968-1 Second Edition 2014-02-01 Milk and milk products - Determination of nitrogen content; AOAC 991.20 Nitrogen (Total) in Milk

Tested with VELP Scientifica DKL 20 Automatic Kjeldahl Digestion Unit (Code S30100210) and UDK 169 Automatic Kjeldahl Analyzer with AutoKjel Autosampler (Code S30200160)



Introduction

A probiotic is a live microbial food ingredient that beneficially affects the host through its effects in the intestinal tract. Probiotics are already widely used to prepare fermented dairy products that are becoming more and more popular in Europe, Japan and USA. These products favorably influence digestive functions and colonic flora. After passage through the stomach and the small intestine, some probiotics survive and become established transiently in the large bowel. The bacterial genera most often used as probiotics are lactobacilli and bifidobacteria, almost exclusively consumed as fermented dairy products such as yogurt.

Protein Determination in Probiotic Drink 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 with digestion block temperature at 400 °C. During the process the organically bonded nitrogen is converted into ammonium sulfate. Alkalinizing the digested solution liberates ammonia which is quantitatively steam distilled and determined by titration.

Sample

Probiotic drink Protein labeled content: 1.3 g/100ml according to the batch production

Sample Digestion

Stir the sample 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 CM (code CT0006650; 3.5 g K₂SO₄, 0.1 g CuSO₄ x 5H₂O Missouri)
- 20 ml concentrated sulphuric acid (96-98%)
- 5 ml of hydrogen peroxide (~ 30%)

Prepare some blanks with all chemicals and without the 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 169 with Autokjel Autosampler** unit by performing the Automatic Check-up and Wash-down in the Menu-System.

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.
VELP titanium condenser ensures that distillate temperature always remains below the safe threshold value (35 °C), as indicated in the IDF 20-1 and ISO 8968-1.

Typical Results on Probiotic Drink

The results are automatically calculated by UDK 169 as a percentage of nitrogen and percentage of proteins. This is "protein" on a total nitrogen basis.

Sample quantity (ml)	Nitrogen %	Protein %
5	0.201	1.283
5	0.203	1.295
5	0.202	1.288
5	0.201	1.283
5	0.201	1.284
5	0.204	1.303
5	0.202	1.288
5	0.200	1.278
5	0.199	1.267
5	0.200	1.274
5	0.200	1.274
5	0.202	1.288
5	0.202	1.286
5	0.202	1.287
5	0.200	1.279
Average ± SD%	0.201 ± 0.001	1.284 ± 0.009
RSD% *	0.686	0.686

Expected Protein Content: 1.3 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 was >99%.

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 169 with AutoKjel Autosampler are:

- High level of precision and reproducibility
- Maximum productivity and full automation
- Worldwide official method
- Reliable and easy method
- Time saving
- Moderate running costs