
N/Protein Determination in Probiotic Drink according to the Dumas combustion method

Reference: **UNI EN ISO 14891**, **FIL-IDF 185**, 2002 "Milk and milk products - Determination of nitrogen content - Routine method using combustion according to the Dumas principle"

Tested with **VELP Scientifica NDA 701 Dumas Nitrogen Analyzer** (Code F30800070)



Introduction

"Probiotics are live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host"
FAO and WHO's definition, 2001

A probiotic is a live microbial food ingredient that can beneficially affect the intestinal tract of the host: after passage through the stomach and the small intestine, probiotics bacteria survive and become established transiently in the large bowel.

Members of the genera *Lactobacillus* and *Bifidobacterium* are already widely used to prepare fermented dairy products due to their probiotic features, and now they are becoming more and more popular in Europe, Japan and USA.

Not only dairy industry finds a deep interest for this kind of product, consumed as yogurt or drinking milk, but also other applications are developing, such in fermented vegetables and meats.

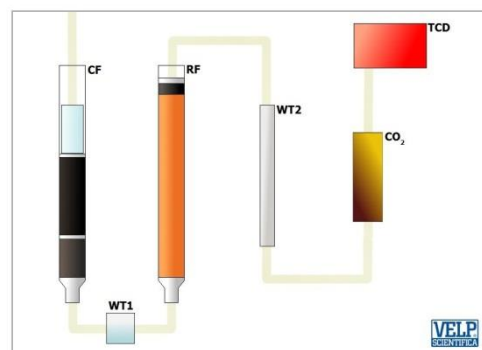
Protein Determination in Probiotic Drink

The Dumas method starts with a combustion furnace (CF) to burn the sample, obtaining elemental compounds.

Water is removed by a first physical trap (WT1 - **DriStep™**), placed after the combustion, and a second chemical one (WT2). Between the two, the elemental substances pass through a reduction furnace (RF).

The auto-regenerative CO₂ adsorbers (CO₂) let pass only the elemental nitrogen that is detected by the **LoGas™** innovative Thermal Conductivity Detector (TCD) with no requirement for a reference gas.

The NDA 701 is controlled via PC through the intuitive **DUMASoft™**.



NDA 701 Preliminary Operations (daily)

Follow the operating manual to start the NDA 701 and check that the following parameters are set:

Temperature Combustion reactor (Code A00000158): 1030 °C

Temperature Reduction reactor (Code A00000226): 650 °C

Flow rate MFC1 He: 190 ml/min

Flow rate MFC2 He: 200 ml/min

Condition the system by testing 2 EDTA standard (Code A00000149) and 3 to 5 empty tin foils (Code A00000153) as Check up.

Verify the calibration curve with one or more tests as Standard by testing the same standard used for the curve creation.

Sample Preparation

Put into the tin foil 70-80 mg of Chromosorb (Code A00000148).

Fill the tin foil with around 200 mg of liquid probiotic milk (~ 200 µl) with an accuracy of 0.1 mg, taken under stirring.

Close the tin foil, obtaining a capsule.

Load the capsule into the autosampler.

Analysis Procedure


Fill the following fields in the database: **Sample name, Weight, Method, Sample type, Calibration number**

The dedicated method "MILK CONDENSED" shows the following parameters:

Protein factor: 6.38

O₂ flow rate: 300 ml/min

O₂ factor: 1.0 ml/mg

Press  to start the analysis.

Analysis time: from 3 minutes for one run.

Typical Results on Probiotic Drink

Sample quantity (mg)	Nitrogen %	Protein %
192.84	0.209	1.334
201.87	0.203	1.292
207.30	0.201	1.285
201.94	0.200	1.277
199.00	0.205	1.309
209.09	0.203	1.298
199.04	0.204	1.304
196.09	0.205	1.310
202.56	0.202	1.289
Average ± SD%	0.204 ± 0.003	1.300 ± 0.017
RSD% *	1.302	1.303

Protein Expected Value: 1.3 %

Protein Factor: 6.38

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

Conclusion

The obtained results are reliable and in accordance with the expected value.

Results have been obtained with the following calibration curve: in a range of 0- 5.9 mg N with 5 measurements of EDTA standard (%N = 9.57) (Code A00000149).

Benefits of Dumas combustion method are:

- High productivity, non-stop performance
- Time saving, few minutes required
- Moderate running costs
- Totally unsupervised, fully automated
- Omission of harsh and toxic chemicals
- Eco-friendly, low amount of residues and wastes

Several organizations working with standardization and recommendation of chemical methods have approved combustion methods for the determination of nitrogen.

Thanks to development in sophisticated instrumentation, the Dumas principle became a practical alternative for the determination of nitrogen in milk derived products.