

Nitrogen and Protein Determination in Commercial Whole Liquid Milk Kjeldahl vs Dumas

Tested with **VELP Scientifica NDA 702 Dumas Nitrogen Analyzer** (Code F30800080), **DKL 20 Automatic Digestion Unit** (Code S30100210) and **UDK169 Automatic Kjeldahl Distillation & Titration System** (Code F30200165)

Dumas Reference: **UNI EN ISO 14891, FIL-IDF 185, 2002** "Milk and milk products - Determination of nitrogen content"

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



Introduction

Whey protein helps improve nutrition. Moreover, it seems they can exert a positive effect on the immune system. Their intake is proposed to improve athletic performance, as an alternative to milk in case of lactose intolerance, instead of artificial milk or to supplement it and in many other cases. In general, milk and dairy products play a role of primary importance in human food and are essential to the balance and the adequacy of the food ration.

The milk composition, as proteins, is an important parameter for its quality evaluation. The protein fraction includes caseins (80%) and whey proteins (20% such as lactalbumin, and lactoglobulin) and it is of interest, not only from a nutritional point of view but also from a technological one. Milk proteins have particular chemical properties which contribute to creating typical sensory features of dairy products (i.e., cheese and yoghurt), influencing whole food processing. Nitrogen determination is commonly carried out using the Kjeldahl method. Here we want to give a comparative of this classic analysis and the Dumas technique elemental analysis testing a commercial whole liquid milk.

Velp provide systems to perform both analyses:

- NDA 702 elemental analyzer to follow the Dumas method
- A system composed of DKL20, SMS scrubber, JP Pump (for automatic digestion) and UDK169 (for distillation and automatic titration) to follow the Kjeldahl one

Kjeldahl - Dumas: an overview of the methods

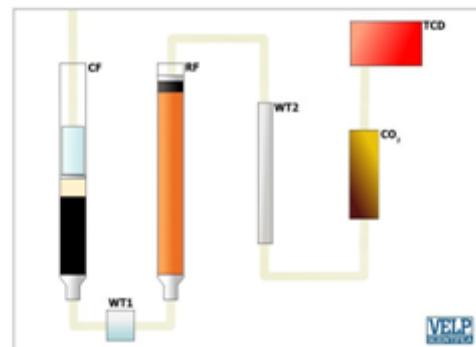
The modern Kjeldahl method consists of a procedure of catalytically supported mineralization of organic material in a boiling mixture of sulphuric acid and sulphate salt at digestion temperatures higher than 400 °C. During the process, the organically bonded Nitrogen is converted into ammonium sulphate.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

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 by a second chemical one (WT2). Between the two, the elemental substances passed 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 702 is controlled via PC through the intuitive **DUMASoft™**.



Sample Preparation

The sample, commercial whole liquid milk, is liquid. So, about 100 mg of Super adsorbent powder (code A00000317) was loaded into the tin foil (Code A00000153) before the sample. It absorbs the liquid and helps the operator in handling and closing the tin foil. The tin foil was carefully closed by hands, obtaining a small capsule, to be loaded into the autosampler.

Dumas Method - NDA 702 Procedure

Follow the operating manual to start the NDA 702 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 Helium: 190 ml/min

Flow rate MFC2 Helium: 220 ml/min

Condition the system by testing 2 to 5 empty tin foils (Code A00000153) as checkup and 2 EDTA standards (Code A00000149) to check the calibration curve accuracy.


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

Create a new customizable method with the following parameters:

Sample weight: ~ 200mg

Protein factor: 6.38

Method: Liquid Milk

Press  to start the analysis

Analysis time: from 4 minutes for one run.

Results have been obtained with the following calibration curve: in a range of 1 – 7 mg N with 5 measurements of EDTA standard (N% = 9.47) (Code A00000149)

Kjeldahl Method: UDK169 Procedure

Sample Digestion

Put about 5ml of sample into a 250 ml test tube (Code A00000144). In each test tube add:

- 2 catalyst tablets KjTabs VCM (code A00000274)
- 2 antifoam tablets KjTabs VS (Code A00000283)
- 20 ml concentrated sulphuric acid (96-98%)
- 5 ml hydrogen peroxide H₂O₂ (30-35%)

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 the digestion phase.

Digest the samples, setting the following method: "01 – Milk and derived products".

Distillation and Titration

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

Condition the UDK 169 unit by performing the Automatic checkup in Menu-System and a Wash down.

Distil the samples by selecting the method "**01-milk and derived products**" in the operating manual.

In UDK 169 settings, set as a unit of measure mgN and %N for the final result and as sample quantity "g".

Distillation and Titration analysis time: from 5 minutes for one test.

Results

Whole liquid milk sample nutritional info: Energy 278kJ/66kcal; Fat 3.6%; Carbohydrates 5.1%; **Protein 3.3%**; Salt 0.1%

Whole liquid milk

	TN% Average ± SD%*	RSD%**	Protein% Average ± SD%*	RSD%**
NDA 702	0.517 ± 0.002	0.537	3.298±0.017	0.534

UDK 169	0.523 ± 0.001	0.221	3.337±0.008	0.242
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(*) Average ± standard deviation

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

Conclusion

The accuracy of both systems has been checked with standards before starting the analysis and can therefore be considered compliant. All the data obtained are acceptable and comparable with the expected value.

The elemental analyzer can easily determine the nitrogen content on commercial whole liquid milk samples. The results are reliable and comparable to the Kjeldahl results. The repeatability of the results is also very good (RSD ≤ 1 %) and the data confirm the complete combustion of the sample with no memory effect observed. NDA 702 allows to work with a high throughput since the analysis time is very low (4/5 minutes), and the operator time on the machine is drastically reduced. VELP Scientifica NDA 702 Dumas Nitrogen Analyzer is the perfect response to simple, fast and precise nitrogen/protein determination.

These results prove the validity of the two methods (Kjeldahl and Dumas). Furthermore, with the Dumas method, using NDA 702, it is possible to obtain the expected result in less time than with the Kjeldahl method. Additionally, the preparation of the sample does not require the use of aggressive reagents, preserving the safety of the operator. Finally, the Kjeldahl method offers optimal results and represents the ideal solution for those who need to carry out these analyses using this method.

In addition, connect the [UDK 169](#) and the [NDA 702](#) to the exclusive [VELP Ermes Cloud Platform](#) to improve your laboratory experience.