APPLICATION NOTE

F&F-D-001-2020/A1

NPN and NCN Determination in Milk

according to the Dumas combustion method

Reference: ISO 14891:2002 [IDF 185:2002] - Milk and milk products — Determination of nitrogen content — Routine method using combustion according to the Dumas principle.

Literature: “Standardization of procedures for nitrogen fractionation of ruminant feeds”. G. Licitra T.M.Hernandez P.J.Van Soest. *Animal Feed Science Technology*, Volume 57, Issue 4, March 1996, Pages 347-358

Tested with **VELP Scientifica NDA 702 Dumas Nitrogen Analyzer (Code F30800080)**

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NPN AND NCN DETERMINATION IN MILK
DUMAS METHOD

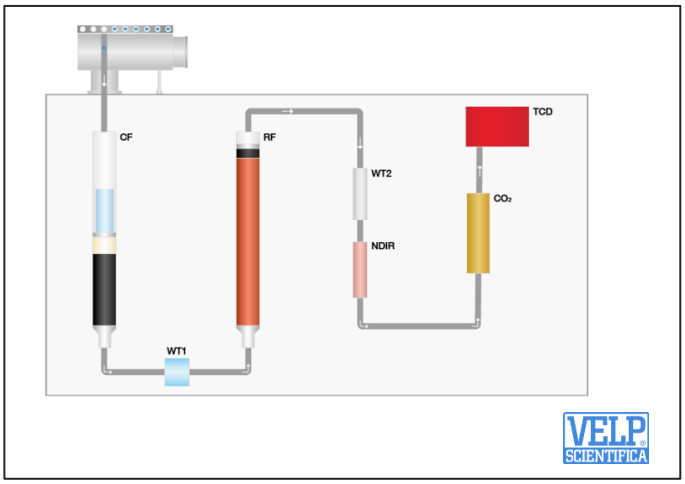
Introduction

Milk is a source of animal protein of high nutritional quality. The nitrogen compounds of milk can be divided in: casein proteins and non-casein nitrogen (NCN). Among this, there are soluble whey proteins (WP), enzymes and non-proteins nitrogen substances (NPN), composed of urea, amino acids, uric acid, creatine, creatinine and ammonia.
Casein is composed of different single proteins, which are linked together with other substances, such as water, enzymes, calcium and phosphorus, and take on a spherical form, named micelle, which have important digestive and also industrial functions, especially in the dairy sector.
Whey contains milk proteins called alpha-lactalbumin and beta-lactoglobulins, which provide a great contribution in terms of branched chain amino acids, much higher than in casein. They are easily digestible by the body, and enter the circulation much faster.
Furthermore, adulterants as NPN are added in milk to increase protein content, thereby increasing the milk quality in fraudulent way. Commercial urea (Sharma et al. 2012) or melamine was added to milk to increase protein content falsely (Liu et al. 2012). So, the determination of NPN is important to discriminate the "true protein content" from "crude protein content".
Using NDA 702, as described below, it's possible to easily and quickly determine the nitrogen content of all the N fractions obtainable with the cited reference method and in accordance with the cited literature.

NPN and NCN Determination in milk according to the Dumas method

Although Kjeldahl is nowadays the most common method for determining nitrogen and protein contents in foods and feeds, Dumas method, shows a high level of precision and reproducibility compared to Kjeldahl method.

The Dumas combustion analysis starts with a combustion (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 traps, the elemental substances pass through a reduction furnace (RF). The auto-regenerative CO_2 absorbers (CO_2) let pass the elemental nitrogen that is detected by the LoGas™ innovative Thermal Conductivity Detector (TCD) with no requirement for a reference gas.



Sample

Liquid whole bovine high-quality milk, pasteurized:

- Protein labeled value: 3.35 g/100 ml
- NCN content from literature: 0.105 %
- NPN content from literature: 0.025 %

Sample preparation

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

Chemicals and materials for separating and filtrating

Trichloroacetic acid solution 20% - 20 g trichloroacetic acid diluted to 100 ml with deionized water
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

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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 casein (NCN) or precipitation of proteins (NPN) and filtration
- Combustion of the filtrate using NDA Elemental Analyzers
- Calculation (see the following formulas)

1- Procedure for Non-casein Nitrogen

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 (VELP 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.

2- Procedure for Non-protein Nitrogen

Place 20 ml of milk, previously thermostated at 20 °C, in a 50 ml volumetric flask and fill up with trichloroacetic acid solution (20%) to the calibration mark. Let the mixture stand for 30 min. Filter with a filter paper and collect the filtrate.

Dumas method

The NDA 702 Dumas Nitrogen Analyzer performs the quantitative analysis of nitrogen by combustion, using helium as carrier gas.
The filtrates were weighed directly in a **Silver foil (Code A00000371)** on the balance using a Pasteur pipette with superabsorbent powder.
Silver foil capsules have been made using the Mold for tin foils (Code A00000262).
Open silver foil cups containing the samples have been left to dry on the Kit for TOC/sample preparation (Code TA00000378) for 30 minutes at 105 °C.
The silver foil was carefully closed by hand, obtaining a little capsule and loaded into the autosampler.
All the parameters of the Dumas method are shown in the table below.

Typical Results

The instrument has been conditioned analysing 2 EDTA standards and 3 to 5 empty tin foils as check-up. For the calibration of the instrument, a glycine standard solution (0,075 N%) has been made dissolving 1g of Glycine (18,66 %N) into 250 ml of deionized water.

Sample ID	Filtrate quantity (mg)	Average N fractions % ± SD% (*)	RSD% (**)
DA147 – Non-casein Nitrogen	300	0,1363 ± 0,0003	0,227
DA148 – Non-protein Nitrogen	300	0,0494 ± 0,0001	0,259

(*) average of 3 runs ± standard deviation of Nitrogen percentage (g/100 g)
(**) Relative Standard Deviation = (SD/average)*100
NOTE: The results refer only to the material received and tested.

The results of the *non-casein nitrogen (NCN) and non-protein nitrogen (NPN)* are calculated as percentage of nitrogen, using mg N, obtained by the calibration curve, divided by the volume of filtrate (*V filtrate*), by 1000 (conversion g to mg) and by dilution factor (G milk/V sol = 20 g / 50 ml = 0.4).

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They are based on the following formula:

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

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 = volume used to perform each analysis (ml)*

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

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 = volume used to perform each analysis (ml)*

**To obtain V filtrate (ml), the sample weight of each test (about 300 mg) has to be converted in ml, considering the filtrate density (NCN filtrate density: 1056.2 mg/ml; NPN filtrate density: 1065.3 mg/ml).*

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.

The VELP NDA 702 ensures reliable results in an easy and fast way. Despite low sample weights, excellent reproducibility is achieved and the data confirm the complete combustion of the sample with no memory effect observed.

The main advantages of VELP Dumas Combustion analyzer:

- Rapid analysis in 3-4 minutes per samples
- Precision and reproducibility guaranteed by the lowest LOD of 0.001 mgN with Helium and the RSD lower than 0.5% (EDTA)
- Low cost per analysis thanks to innovative technology and genuine consumables
- Fast set up of the instrument
- Easy maintenance
- Intuitive software User Interface
- Safety for the laboratory since NDA Series does not require any aggressive chemical
- Connectivity to **VELP Ermes Cloud Platform** for remote Application and Service support, remote access to the instruments, alerts and notifications and much more.

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