APPLICATION NOTE

Non-protein nitrogen determination in milk and milk products - Dumas method

Adapted from the method based on the standard **DIN EN ISO 8968-4:2016** - Milk and milk products - Determination of protein nitrogen content and non-protein nitrogen content and calculation of the actual protein content.

Introduction

C. Gerhardt Devices:
- DUMATHERM N Pro

Additional equipment:
- Analytical balance
- Mixer
- Water bath
- Filtration station
- Fume cupboard

Milk and dairy products contain high-quality proteins that humans can utilise particularly well and use to build up the body's own protein. Milk proteins are not only important in the production of traditional dairy products, but also play an important role in a wide range of food products, such as baby food and in the pharmaceutical sector, due to their diverse functional properties and high nutritional-physiological value. Accordingly, the protein content of milk has a significant role in determining the price.

Milk proteins essentially consist of casein, whey proteins and "non-protein nitrogen" (NPN). NPN is the component of the raw protein that cannot be processed by humans and is therefore distinguished from the so-called real protein or pure protein. NPN is a crucial component of milk composition and comprises various nitrogenous compounds that are not proteins, but are nevertheless of great importance for assessing product quality and safety. NPN is composed of creatine/creatinine, peptides, hippic acids, free amino acids, orotic acid, uric acid, ammonia and urea (urea), with urea making up the largest part. Therefore, to determine the relevant protein content, the NPN content must be subtracted from the protein content with the following calculation:

Pure protein = crude protein - NPN.

In the field of dairy product quality control and nutritional analysis, the accurate determination of non-protein nitrogen (NPN) in milk and dairy products plays a central role. The determination of NPN is relevant because the protein content can be artificially increased by adding other substances with a high nitrogen content.

An example of this is the melamine scandal in China a few years ago - melamine, an industrial chemical, was added to milk powder to increase the protein content. A pure nitrogen determination according to Kjeldahl reaches its limits here and would show a protein content that is too high. However, the determination of NPN is also used to draw conclusions regarding the

quality of the animal feed - based on the results of the NPN/urea analysis, the content or the sequence of the rations can be adjusted to optimise feeding costs, milk production and the reduction of nitrogen waste in the environment.

The method

Sample preparation

The **liquid samples** are transferred to a beaker and heated to a temperature of 38-40 °C in a water bath. The sample to be analysed is then cooled to room temperature with careful mixing and weighed in an Erlenmeyer flask. Trichloroacetic acid is then added to the milk sample and the milk-acid mixture is weighed again. After formation of the precipitate, the contents of the conical flask are filtered and the filtrate is collected in a clean, dry conical flask.
Solid samples are homogenised with a mixer or rotor mill, if necessary, and an appropriate amount of the sample is dissolved in water at 40-50 °C. Trichloroacetic acid is added to form the precipitate, which is filtered off after briefly heating the suspension so that the filtrate can be collected in a clean, dry Erlenmeyer flask. Before weighing, tin foil (e.g. DumaFoil) is tared, 75 mg superabsorbent is weighed in and the sample is weighed in using a disposable syringe.

➔ **Application note**: Due to the low nitrogen content, approx. 400 mg of the filtrate must be weighed in. Here it may be advantageous to use DumaFoilXL to simplify sample handling.

Weighing / Calibration

With a sample weight of approx. 400 mg filtrate, peak areas between 400-900 mVs are achieved, depending on the sample, which corresponds to an absolute amount of nitrogen of approx. 0.08 - 0.18 mg. Therefore, the chosen calibration should cover this working range. For such low nitrogen contents, a THAM solution is usually used, here a THAM solution with 0.05% N nitrogen, which covers the desired working range with weights between 150 mg to 400 mg. The minimum requirement for the correlation factor R2 is a value of ≥ 0.999.

Calculation of results

The non-protein nitrogen content is calculated as a function of the sample weights, the sample-acid mixture, the filtrate and the nitrogen weight in the filtrate.

➔ **Application note**: Use our already prepared Excel spreadsheet for the calculation, which we will be happy to provide you with.

Table 1: Analysis results for NPN determinations Dumas method.

Sample type	Sample quantity filtrate in [mg] +/- 10%	Measured protein content [%]	Standard deviation	Relative standard deviation
Cow's milk	400	0,155	0,005	3,519
Whey isolate	400	2,906	0,102	3,509

Table 2: Example results for cow's milk.

Sample quantity [mg]	Protein factor	N Weight [mg]	NPN Sticksotff [%]	NPN protein [%]
406,254	6,38	0,080	0,026	0,167
404,697	6,38	0,070	0,023	0,147
403,159	6,38	0,077	0,025	0,162
407,195	6,38	0,072	0,023	0,149
404,635	6,38	0,073	0,024	0,154
403,500	6,38	0,074	0,025	0,156
		Average	0,024	0,155
		Standard deviation	0,001	0,005
		RSA [%]	3,519	3,519

Conclusion

For milk and dairy products, the protein content is of major importance for assessing the quality and determining the price. Therefore, it is important for analytics to also determine the "true" protein content in the milk. An NPN analysis with DUMATHERM provides laboratories with an important value for determining the actual protein content.

For more information or other applications, please contact:

application@gerhardt.de