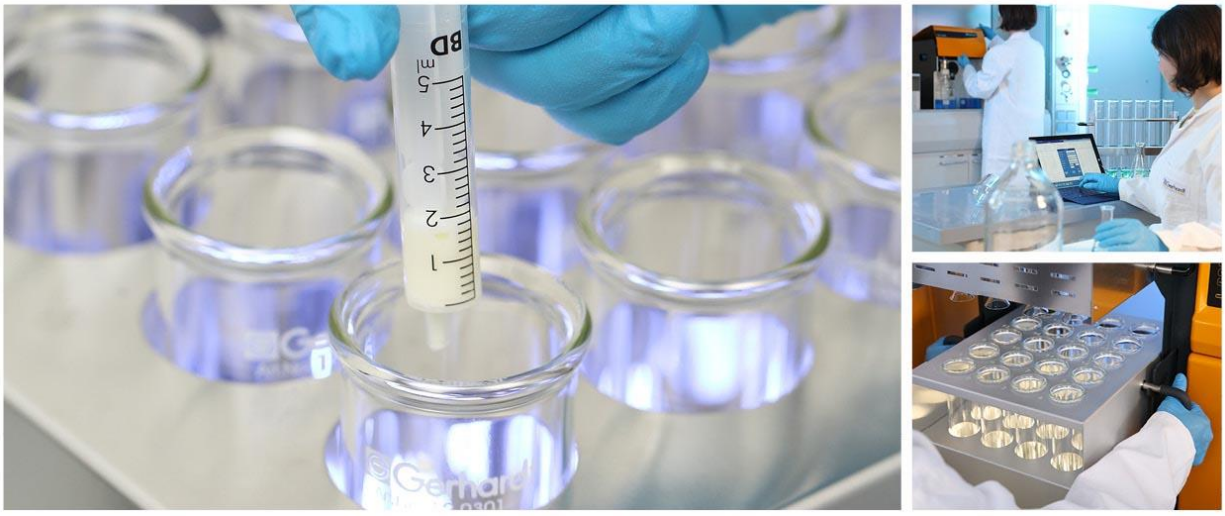
# APPLICATION NOTE

## Non-protein nitrogen determination - NPN - in milk and milk products - Kjeldahl method

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:**
- KJELDALTHERM KT20
- VAPODEST 500
- VACUSOG

**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. The filtrate is weighed by differential weighing with a disposable syringe.

**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. The precipitate is formed by adding trichloroacetic acid, which is filtered off after briefly heating the suspension. The filtrate can be weighed out with a disposable syringe.

The filtrate must be clear and free of particles. If this is not the case, precipitation and filtration are repeated.

### Outcrop

The sample is digested in concentrated sulphuric acid at 410 °C. The filtrate does not tend to foam, but should still be heated carefully and observed. With the official standards, the digestion time is 2.5 hours, whereas with an optimised method, the digestion time can be reduced to about 2 hours.

➔ **Application note**: Shorten the digestion time by placing the samples in a preheated digestion block.

### Distillation and titration

After digestion, the sample is distilled with the addition of $H_2O$ and NaOH in a receiver made of $H_3BO_3$. The endpoint determination is carried out automatically in the VAPODEST 500. The addition of a mixing indicator is not necessary, but can be used for visual control.

### Calculation of results

The non-protein nitrogen content is calculated depending on the previously determined blank value of the noted weights, the weights of the sample, the sample-acid mixture and the filtrate as well as the consumption of the titration solution.

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

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## Analysis results

**Table 1:** Analysis results for NPN determinations with the Kjeldahl method.

| Sample type | Sample quantity filtrate in [mL] +/- 10% | Measured protein content [%] | Standard deviation | Relative standard deviation |
|---|---|---|---|---|
| Cow's milk | 20 | 0,17 | 0,002 | 1,183 |
| Whey isolate | 25 | 4,332 | 0,021 | 0,476 |
| Protein isolate (vegan) | 20 | 2,524 | 0,010 | 0,386 |
| Hard cheese | 10 | 4,733 | 0,023 | 0,486 |

**Table 2:** Example results for whey isolate.

| Sample quantity [mL] | Protein factor | V-VB [mL] | NPN Sticksotff [%] | NPN protein [%] |
|---|---|---|---|---|
| 25 | 6,38 | 7,045 | 0,684 | 4,361 |
| 25 | 6,38 | 6,977 | 0,677 | 4,319 |
| 25 | 6,38 | 6,972 | 0,676 | 4,316 |

| | | |
|---|---|---|
| Average | 0,679 | 4,332 |
| Standard deviation | 0,003 | 0,021 |
| RSA [%] | 0,476 | 0,476 |

## 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. NPN analysis with KJELDATHERM and VAPODEST provides laboratories with an important value for determining the actual protein content.

**For more information or other applications, please contact:**

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