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A compilation of various methods for protein determination in milk, based on the classic determination by Kjeldahl.

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There are only few foodstuffs that are as closely connected to the history of mankind as milk. Domestic cows for milking were kept already in the 5th millennium B.C. and in India cows are considered holy to this day.

Not only does milk taste good, it is also a healthy and nutritious food, in fact one of the most valuable foodstuffs there is; it contains healthy protein for building up muscles, calcium for strong bones and teeth, milk sugar for good digestion and vitamins for a strong immune system. Milk protein is very valuable because it contains a large proportion of essential amino acids and is therefore indispensable for the structure of body cells (such as muscles, organs, skin, hormones, enzymes).

Worldwide agricultural milk production provides 609 million tonnes per annum. The largest milk producers are India, the USA and Russia. The European Union (EU-15) produces around 120 million tonnes per year; it is thus the biggest market for milk products. [1] [2]

The consumption of milk is rising strongly on a worldwide basis though primarily in the form of processed dairy products. The food industry processes milk in numerous ways and into many different products, from cheese and bakery products or ice cream production through to uses in meat processing or in the production of ready meals. [3]

Since the overall protein content plays an important role both for the payment of milk delivered and for determining the breeding value of cows, it is subject to regular checks.

For many years, the reference method for determining the protein content has been the Kjeldahl method [4]. In this method the nitrogen content of a sample is determined and then multiplied by a specific factor (for milk 6.38) to obtain the protein content. However, as milk also contains other sources of nitrogen (Non-Protein Nitrogen compounds = NPN), these are also included and show up as protein.

In order to eliminate this error the calculation of the payment for milk was switched from total protein to pure protein in some countries.



Picture 1:
KjeldFlex
K-360



Picture 2: Sampler
System K-370/K-371

Introduction

Protein content in milk:

The total protein of milk consists of approx. 94% pure protein (approx. 3.1g/100g) and approx. 6% NPN (approx. 0.2g/100g). The average total protein content of milk is 3.3 %.

Total protein

Total protein consists of protein and other, Non-Protein Nitrogen compounds (NPN), which nevertheless are included in the count.

Pure protein

Pure protein consists of casein and whey protein.
(Pure protein = total protein minus NPN)

NPN (Non-Protein-Nitrogen compounds)

NPN is composed mainly of urea and other N-compounds (e.g. amino-nitrogen, peptide-nitrogen, creatine, keratine and ammonia) [5].

Methods

Each laboratory applies different criteria for their method of determination (using classic methods that are officially established under e.g. §35 LMBG, § 64 LFGB, AOAC, ISO, EPA and DIN, or other methods where the official ones are not required where considerations are those of saving chemicals, fast processing times and environmental protection), which is why some modified methods were adopted that derive from the original method.

Kjeldahl process (official method)

Process:

When determining the protein according to Kjeldahl, the milk sample is first treated with concentrated H_2SO_4 , which leads to the formation of ammonium sulphate. Through the alkalisation with NaOH, the ammonia is displaced from the ammonium sulphate and over-distilled into a boric acid receiver via steam distillation. This is then titrated with an HCl or H_2SO_4 titrating solution [6].

NPN determination

The first step in the NPN determination is to precipitate the protein with trichloroacetic acid. Then the filtrate is analysed in accordance with the Kjeldahl method [7].

Direct distillation of milk

Direct distillation is a simple and above all fast method for determining the protein content since digestion is omitted here [8].

Process:

Put 10 ml of the milk sample into the sample tube. Just before starting distillation, add 20 ml barium chloride (10%) to the sample; then proceed in the same way as in the official Kjeldahl method.

The milk sample is heated in an alkaline solution and thereby releases ammonia. The largest part of the ammonia is formed through fast hydrolysis of the protein components glutamine and asparagine. This breaking-up process is completed within a few minutes. In addition, a small quantity of ammonia is produced through the complete destruction of other amino acids, but the destruction is so slow that it does not affect the process.

This results in a factor (conversion factor) which needs to be determined in order to be able to simply determine the nitrogen or protein content.

With the direct distillation method it is possible to determine the protein content within about 10 minutes.

Kjeldahl process with H_2SO_2 digestion

The digestion with H_2O_2 is particularly suitable for heavily foaming samples. However, in order to be able to carry out this digestion one needs a special suction module (picture 3) to be able to add H_2O_2 .

The funnels of the suction module are equipped with glass frits that facilitate an even inflow of H_2O_2 .



Picture 3: H₂O₂ suction module

Advantages of the H₂O₂ digestion:

- Approx. 50% reduced digestion time
- No foaming of samples during the digestion process
- An environment-friendly process as no heavy metals are used
- Only 69% of H₂SO₄ are used instead of 98% H₂SO₄

Process:

Put 5 g of milk into the sample tubes and then add 10 ml of H₂O₂ (30%) and 30 ml of H₂SO₄ (69%). Connect the H₂O₂-suction module with the

rack. The lots of 20 ml of H₂O₂ are added after the first 10 minutes of the digestion process [9].

'Micro'-Kjeldahl method

The Micro-method is carried out in the same way as the official Kjeldahl method.

However, as this method requires a much smaller volume of milk in the sample (about 1.8 mL), the quantities of chemicals used are less and the digestion time is significantly reduced compared to the official method [10].

Table 1: equipment

	Official method	Micro-method	H ₂ O ₂ digestion	Direct distillation	NPN
Digestion	K-438	K-435	K-435 with H ₂ O ₂ suction tube	-	K-438
Scrubber	B-414	B-414	B-414	-	B-414
Distillation	K-370/371	K-370/371	K-370/371	K-360	K-370/371

The above units were used for the processes described in this document. It is of course also possible to use other distillation and digestion devices from Büchi.

Table 2: digestion parameters

Official method		Micro-method		H ₂ O ₂ digestion		Direct distillation		NPN	
step	time	step	time	step	time	step	time	step	time
230°C	15 min	10	10 min	10	10 min (+20ml H ₂ O ₂)	-	-	420 °C	120 min
300°C	40 min	8.5	80 min	10	20 min	-	-		
350°C	40 min	-	-	-	-	-	-		
420°C	60 min	-	-	-	-	-	-		
cooling	30 min	cooling	30 min	cooling	30 min	-	-	cooling	
total time	3h 05 min		2 h		55 min		0 min		30 min

Table 3: distillation parameters

Distillation	Official method	Micro-method	H ₂ O ₂ digestion	Direct distillation	NPN
water	50 ml	20 ml	50 ml	50 ml	50 ml
NaOH 32 %	90 ml	35 ml	90 ml	70 ml	90 ml
react. time	5 s	5 s	5 s	5 s	5 s
dist. time	300 s	200 s	240	360 s	240 s
Steam power	100%	100%	100%	100%	100%

Table 4: titration parameters

Titration	Official method	Micro-method	H ₂ O ₂ digestion	Direct distillation	NPN
boric acid (pH 4.65)	50 ml (4%)	40 ml (2%)	60 ml (4%)	60 ml (4%)	60 ml
titr. solution	HCl 0.1 mol/L	HCl 0.05 mol/L	H ₂ SO ₄ 0.25 mol/L	H ₂ SO ₄ 0.1 mol/L	HCl 0.01 mol/L
titr.method	standard	standard	standard	-	standard
type	end point	end point	end point	end point	end point
pH	4.65	4.65	4.65	4.65	4.65

For determining low nitrogen concentrations (< 10 mg N absolute) it is recommended to use a low concentration of boric acid (e.g. 2%, see Micro-method) in order to be able to detect the turning point better.

The weight/volume of the sample and the titration solution concentration should be selected such that about 5 to 20 mL of titration solution will be used.

Table 5: results

	Official method	Micro-method	H ₂ O ₂ digestion	Direct distillation	NPN
sample volume [mL]	5	1.8	5	10	10
type of milk	semi-skimmed milk (UHT)	whole milk (UHT)	skimmed milk (UHT)	semi-skimmed milk (UHT)	whole milk (UHT)
declared protein content of milk	3.0 g / 100 mL	3.0 g / 100 mL	3.2 g / 100 mL	3.2 g / 100 mL	3.0 g / 100 mL
number of samples	8	3	12	10	4
RSD	0.43 %	0.41	1.25 %	1.00 %	2.4 %
result	3.18 %	3.12 %	3.33 %	3.29 %	0.14 %
time input (digestion & determination)	3h 15min	2h 10min	1h 5min	10 min	2h 40min

**Comparative measurements with the official method
(The determination was carried out with the same milk as in the method above)**

sample volume [mL]	No comparison necessary as this is the official method	5	5	5	-
number of samples		4	12	12	-
RSD		0.14 %	0.66 %	0.71	-
result		3.14 %	3.36 %	3.30 %	-

As the declaration on the milk packet is stated in g/mL, the declared value cannot be compared directly with the results obtained (g/g = %). So that the results could nevertheless be shown in comparison, the different methods were also compared with the official method.

Conclusion

As can be seen from the above table, the 'optimised' methods can also be used to obtain reproducible results. This means that these can be used to great benefit as alternative methods for routine laboratory processes.

All methods presented here produce very good results. Laboratories may therefore choose the method that suits their requirements best, for example the Micro-Kjeldahl method to reduce the use of chemicals as much as possible, the H₂O₂ digestion in order to achieve a faster and yet accurate result or the direct distillation method in order to achieve results as quickly as possible. However, where it is mandatory to employ an official method (e.g. AOAC, DIN) it is not possible to use the 'optimised' methods.

Source:

- [1] http://de.wikipedia.org/wiki/Milch#Kritische_Informationen_zu_Milchkonsum
- [2] FAO
- [3] http://de.wikipedia.org/wiki/Milch#Kritische_Informationen_zu_Milchkonsum
- [4] <http://jds.fass.org/cgi/reprint/75/11/3218.pdf>
- [5] Source: <http://jds.fass.org/cgi/reprint/75/11/3218.pdf>
- [6] Büchi Application Note: K-438-K-370_371-003
- [7] Büchi Application Note: K-438-K-370_371-006 V1.0
- [8] Büchi Application Note: K-360-002 V1.0
- [9] Büchi Application Note: K-435-K-370-371 V1.0
- [10] Büchi Application Note: K-435-K-370-005 V1.0

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