best @buchi

www.buchi.com Information Bulletin Number 61/2011

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

en

Author: Michaela Buffler, Jürgen Traunig
BÜCHI Labortechnik AG,
Meierseggstrasse 40,
9230 Flawil, Switzerland

There are only few foodstuffs that are as closely connected to the history of mankind as milk. 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 678 million tonnes per annum [1]. The largest milk producers are India, the USA and China [2]. The European Union (EU-27) produces around 148 million tonnes per year; it is thus the biggest market for milk products [3]. 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 [4].

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 [5]. 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: KjelFlex K-360

Picture 2: Scrubber B-414 SpeedDigester K-439

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.

NPN (Non-Protein-Nitrogen compounds)

The non-protein nitrogen (NPN) fraction is composed of urea and other low molecular weight nitrogen containing compounds such as creatine and creatinine. About 50% of the NPN in milk is urea [6].

Pure protein

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

Casein

Caseins are the predominant proteins that account for nearly 80 % of proteins in cow milk that is not attained into the whey and so will be processed to e.g. cheese.

Methods

Each laboratory applies different criteria for their method of determination. Some laboratories are using classic methods that are officially established under e.g. §64 LFBG, AOAC, ISO, EPA and DIN, whereas others are preferred by considering fast processing times, saving chemicals and environmental protection. That is why some modified methods were adopted and derive from the original method.

Kjeldahl process (Standart 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 alkalinisation 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 titration solution [7].

Casein determination

The casein is precipitated from a test portion by the addition of acetic acid solution. Then the filtrate is analysed in accordance with the Kjeldahl method [8].

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 [9].

Direct distillation of milk

Direct distillation is a simple and above all fast method for determining the protein content since digestion is omitted here [10].

Process:
Put the sample into the sample tube. Just before starting distillation, add a portion of barium chloride solution to the sample and then proceed in the same way as in the standard Kjeldahl method.
Before measuring milk samples it is necessary to determine the conversion factor (ratio between total protein nitrogen and ammonia nitrogen) to calculate the protein content taking the consumption of titrant into account.

Kjeldahl process with H_2O_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 guarantee an even inflow of H_2O_2.

Picture 3: SpeedDigester K-439 with H_2O_2 suction module

Advantages of the H_2O_2 digestion:

- Approx. 75% reduced digestion time
- No foaming of samples during the digestion process
- An environment-friendly process as no heavy metals (catalyst) are used

Process:
Add the sample and a portion of sulfuric acid into a sample tube. Connect the H_2O_2-suction module with the rack. During the digestion add two portions of hydrogen peroxide through the capillary funnel [11].

'Micro'-Kjeldahl method
The Micro-method is carried out in the same way as the standard 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 standard method [12].

Table 1: equipment

	Standard method	Micro-method	H_2O_2 digestion	Direct distillation	NPN	Casein
Digestion	K-438	K-439	K-439	-	K-439	K-439
Scrubber	B-414	B-414	B-414	-	B-414	B-414
Distillation	K-370/371	K-370/371	K-370/371	K-360	K-360	K-360

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 BUCHI.
The methods using the K-439 can also be carried out with the K-436 / K-425. The parameters are shown in the corresponding Application note (www.buchi.com).

Table 2: digestion parameters

Standard method		Micro-method		H_2O_2 digestion		Direct distill.		NPN		Casein	
temp.	time	temp.	time	temp.	time	temp.	time	temp.	time	temp.	time
420°C	120 min	550°C	12 min	450°C	20 min	-	-	550°C	35 min	550°C	35 min
-	-	490°C	48 min	480°C	10 min	-	-	490°C	45 min	490°C	45 min
total time	120 min	-	60 min	-	30 min	-	-	-	80 min	-	80 min

Table 3: distillation parameters

Distillation	Standard method	Micro-method	H_2O_2 digestion	Direct distillation	NPN	Casein
water	50 ml	20 ml	50 ml	50 ml	80 ml	80 ml
NaOH 32 %	60 ml	35 ml	90 ml	70 ml	80 ml	90 ml
react. time	5 s	5 s	5 s	5 s	5 s	5 s
dist. time	300 s	200 s	240	360 s	240 s	240 s
steam power	100%	100%	100%	100%	100%	100%

Table 4: titration parameters

Titration	Standard method	Micro-method	H_2O_2 digestion	Direct distillation	NPN	Casein
boric acid (pH 4.65)	50 ml (4%)	40 ml (2%)	50 ml (4%)	60 ml (4%)	50 ml (2%)	50 (4%)
titr. solution	HCl 0.1 mol/L	H_2SO_4 0.05 mol/L	H_2SO_4 0.1 mol/L	H_2SO_4 0.1 mol/L	HCl 0.01 mol/L	HCl 0.1 mol/L

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/NPN) in order to be able to detect the end point better.
The weight/volume of the sample and the titration solution concentration should be selected such that about 3 to 20 mL of titration solution will be used.

Table 5: results

Sample results	Standard method	Micro-method	H_2O_2 digestion	Direct distillation	NPN	Casein
sample volume	2 g	1.8 g	5 g	10 mL	10 g	20 g
type of milk	whole milk	whole milk	whole milk	partial skimmed milk	whole milk	whole milk
declared protein content	3 g/100 mL	3 g/100mL	3 g/100mL	3.2 g/100mL	-	-
number of samples	4	4	4	10	4	4
result	3.43 %	3.40 %	3.13 %	3.29 g/100mL	0.025 %	2.65 %
rsd	0.39	0.59	0.20	1.00	0.85	0.19

The results determined with the different methods are not comparable because different milk batches were used.

Table 6: recovery of tryptophan

Recovery of tryptophan	Standard method	Micro-method	H_2O_2 digestion	Direct distillation	NPN	Casein
recovery	99.5	99.1	99.0	-	-	-
rsd	0.49	0.41	0.04	-	-	-
number of samples	4	8	4	-	-	-

Tryptophan is used for the verification of the method [DIN].
The verification with tryptophan of the methods „direct distillation", „NPN" and „casein" is not possible.

Conclusion

As can be seen from the above tables, the 'optimized' 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 H_2O_2 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 'optimized' methods.

Source:

[1] http://www.thedairysite.com/articles/1755/milk-and-milk-products-a-global-market-analysis
[2] http://www.indexmundi.com/agriculture/?commodity=milk&graph=production
[3] http://www.milchindustrie.de/de/eu/agrarpolitik/quote/
[4] http://de.wikipedia.org/wiki/Milch
[5] DIN EN ISO 8968-1:2001
[6] http://www.cpdmp.cornell.edu/cpdmp/pages/publications/pubs/tpfact.pdf
[7] BUCHI Application note: 063/2011
[8] BUCHI Application note: 051/2010
[9] BUCHI Application note: 050/2010
[10] BUCHI Application note: K-360-002 V1.0
[11] BUCHI Application note: 054/2010
[12] BUCHI Application note: 031/2010

BÜCHI Labortechnik AG
CH – 9230 Flawil 1
T +41 71 394 63 63
F +41 71 394 65 65
buchi@buchi.com
www.buchi.com

BUCHI UK Ltd.
GB – Oldham OL9 9QL
T +44 161 633 1000
F +44 161 633 1007
uk@buchi.com
www.buchi.co.uk

BUCHI Hong Kong Ltd.
HK – Central
T +852 2389 2772
F +852 2389 2774
china@buchi.com
www.buchi.com.cn

Nihon BUCHI K.K.
JP – Tokyo 110-0008
T +81 3 3821 4777
F +81 3 3821 4555
nihon@buchi.com
www.nihon-buchi.jp

BUCHI Korea Inc
KR – Seoul 153-782
T +82 2 6718 7500
F +82 2 6718 7599
korea@buchi.com
www.buchi.com

BÜCHI Labortechnik GmbH
DE – 45003 Essen
Freecall 0800 414 0 414
T +49 201 747 490
F +49 201 237 082
deutschland@buchi.com
www.buechigmbh.de

BÜCHI Labortechnik GmbH
NL – 3340 AC
Hendrik-Ido-Ambacht
T +31 78 684 94 29
F +31 78 684 94 30
netherlands@buchi.com
www.buchi.nl

BUCHI Shanghai
CN – 200052 Shanghai
T +86 21 6280 3366
F +86 21 5230 8821
china@buchi.com
www.buchi.com.cn

BUCHI India Private Ltd.
IN – Mumbai 400 055
T +91 22 667 18983 / 84 / 85
F +91 22 667 18986
india@buchi.com
www.buchi.in

BUCHI Canada Ltd.
CA – Thornhill, Ontario L4J 6Z2
T +1 416 277 7407
F +1 905 764 5139
canada@buchi.com
www.mybuchi.com

BUCHI Sarl
FR – 94656 Rungis Cedex
T +33 1 56 70 62 50
F +33 1 46 86 00 31
france@buchi.com
www.buchi.fr

BUCHI Italia s.r.l.
IT – 20090 Assago (MI)
T +39 02 824 50 11
F +39 02 57 51 28 55
italia@buchi.com
www.buchi.it

BUCHI (Thailand) Ltd.
TH – Bangkok 10600
T +66 2 862 08 51
F +66 2 862 08 54
bacc@buchi.com
www.buchi.com

PT. BUCHI Indonesia
ID – Tangerang 15321
T +62 21 537 62 16
F +62 21 537 62 17
indonesia@buchi.com
www.buchi.com

BUCHI Corporation
US – New Castle,
Delaware 19720
Toll Free: +1 877 692 8244
T +1 302 652 3000
F +1 302 652 8777
us-sales@buchi.com
www.mybuchi.com

We are represented by more than 100 distribution partners worldwide. Find your local representative at

www.buchi.com

Quality in your hands