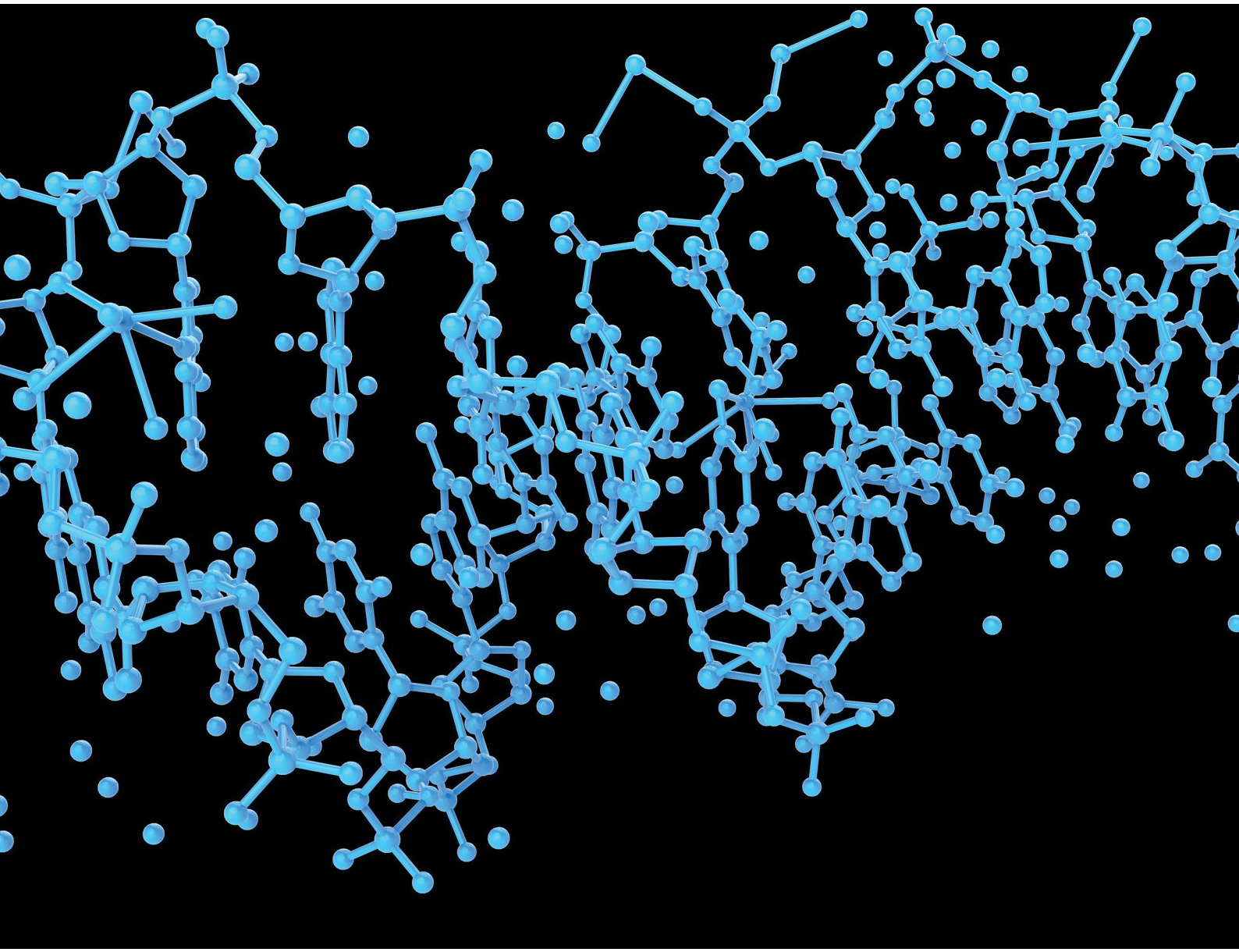
Nitrogen and protein content measurement and nitrogen to protein conversion factors for dairy and soy protein-based foods: a systematic review and modelling analysis



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FINAL DRAFT

NITROGEN AND PROTEIN CONTENT MEASUREMENT AND NITROGEN TO PROTEIN CONVERSION FACTORS FOR DAIRY AND SOY PROTEIN-BASED FOODS: A SYSTEMATIC REVIEW AND MODELLING ANALYSIS

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ABBREVIATIONS

AA	amino acid
AACC	American Association of Cereal Chemists
AOCS	American Oil Chemists' Society
BCA	bicinchoninic acid
CCNFSDU	Codex Committee on Nutrition and Foods for Special Dietary Uses
CI	confidence interval
CV	coefficient of variation
FAO	Food and Agriculture Organization of the United Nations
FSTA	Food Science and Technology Abstracts
GRADE	Grading of Recommendation, Assessment, Development and Evaluation
HCl	hydrochloric acid
HPLC	high-performance liquid chromatography
IDF	International Dairy Federation
ISO	International Organization for Standardization
JEMNU	Joint FAO/WHO Expert Meetings on Nutrition
MIR	mid-infrared reflectance
NH3	ammonia
NIR	near infrared reflectance
NPCF	nitrogen to protein conversion factor
NPN	non-protein nitrogen
PICO	population, intervention, comparator, outcome
PN	protein nitrogen
RP-HPLC	reverse-phase high-performance liquid chromatography
SD	standard deviation
TN	total nitrogen
UHPLC	ultra-high-performance liquid chromatography
UNSCN	United Nations System Standing Committee on Nutrition
UV	ultraviolet
WHO	World Health Organization

Amino acids

ala	alanine	leu	leucine
arg	arginine	lys	lysine
asn	asparagine	met	methionine
asp	aspartate	phe	phenylalanine
cys	cysteine	pro	proline
gln	glutamine	ser	serine
glu	glutamate	thr	threonine
gly	glycine	trp	tryptophan
his	histidine	tyr	tyrosine
ile	isoleucine	val	valine

1. INTRODUCTION

The World Health Organization (WHO) recommends that infants be exclusively breastfed for the first 6 m onths of life, and thereafter receive nutritionally adequate and safe complementary foods, while continuing to breastfeed for up to 2 years or beyond (WHO, 2013). There are circumstances, however, in which the use of breast-milk substitutes (i.e. infant formula) may be acceptable (FAO/WHO, 2016a). In addition, "follow-up formula" is sold and consumed by older infants and young children. Dairy-based ingredients and soy-based ingredients are used as a source of protein in such formula. The Codex Alimentarius Commission has established standards for infant formula (FAO/WHO, 1981) and follow-up formula (FAO/WHO, 1987), including a range of acceptable protein levels (i.e. a minimum and maximum amount of protein per 100 kcal). The method for calculating protein content of food products currently described in the standards is to measure nitrogen content and then convert to protein using a conversion factor of 6.25 for both dairy-based and soy-based ingredients, unless a scientific justification is provided for the use of a different conversion factor for a particular product.

Nitrogen to protein conversion factors (NPCFs) allow for the estimation of protein content in food samples from the amount of nitrogen in the sample, based on two assumptions: that most of the nitrogen is associated with amino acids and that most of the amino acids in foods are associated with protein. The accuracy of the estimation depends on the value of the conversion factor. A value of 6.25 is applied for measuring protein content in most foods and food ingredients, again based on two assumptions: that proteins contain about 16% nitrogen by weight (i.e. of the total mass of a protein, 16% is nitrogen), and that all nitrogen in food is derived from protein. However, using the same conversion factor for all protein sources can introduce errors that lead to significant overestimation or underestimation of the actual protein content of most foods. Hence, a default value of 6.25 may not be an appropriate conversion factor for all protein sources; instead, specific values should be considered for different foods and food ingredients.

Across Codex standards, there is no single universally agreed NPCF for dairy and soy. The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) has recently raised the question of the appropriate NPCFs to be used for milk and soy protein in infant formula and follow-up formula. To this end, CCNFSDU sought advice from the Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Expert Meetings on Nutrition (JEMNU)[1] – and requested that JEMNU be convened to review the evidence and develop evidence-informed guidance regarding NPCFs.

This review was commissioned by FAO and WHO to serve as a background document for JEMNU. The objective was to assess known conversion factors for dairy-based and soy-based ingredients through a systematic review of the literature, including methods and approaches used for the determination of NPCFs, with the aim of supporting the selection of appropriate[2] conversion factors for dairy-based and soy-based ingredients used in infant formula and follow-up formula.

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[1] JEMNU was established in 2009 to provide scientific advice to the committees of the Joint FAO/WHO Food Standards Programme (i.e. Codex Alimentarius Commission) or Member Countries, in an independent, timely and cost-effective manner.

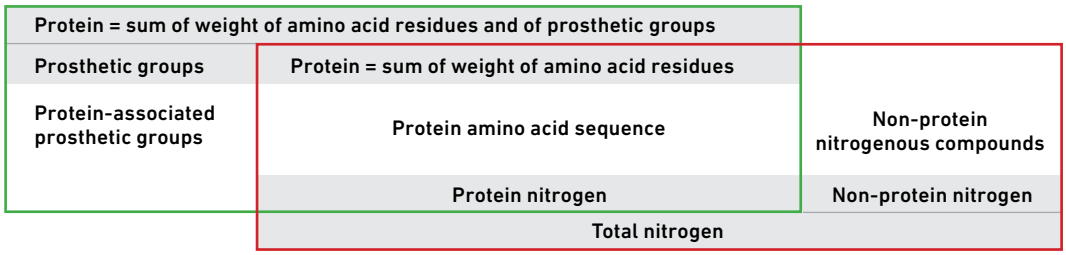
[2] In the context of this document, "appropriate" refers solely to "most likely to provide the best estimate of the actual protein content".

2. BACKGROUND

2.1 Protein, protein nitrogen and non-protein nitrogen in foods

In foods, nitrogen is mainly associated with protein as protein nitrogen, but it can also be present in other non-protein food components as non-protein nitrogen (**Fig. 1**).

Fig. 1 Protein and nitrogen in foods



Proteins and peptides are macromolecules that comprise covalently bonded amino acid residues organized as linear polymers. The sequence of the amino acids in a protein or peptide determines the properties of the molecule. Proteins are large molecules that commonly exist as folded structures with a specific conformation, whereas peptides are smaller and may comprise only a few amino acids.

Proteins are defined biochemically by their amino acid residue content and by the sequence of those amino acids, which forms the primary structure:

- Up to 20 different amino acid residues are included in the composition and sequence of a protein; proteins in food or food ingredients differ in terms of the amount of each amino acid residue that they contain.
- Proteins differ in their amino acid residue composition, and amino acid residues differ in molecular weight. The weight of a particular amino acid residue in the protein fraction of food is derived from the molecular weight of that residue and the frequency with which it occurs.
- Many proteins are modified post-translationally (e.g. through phosphorylation or glycosylation); the prosthetic groups added to the constituent amino acids in such modifications can change the characteristics of the protein and increase its mass. Prosthetic groups vary in weight, from 14 Da for methylation to over 200 Da for fatty acylation, prenylation, nucleotide conjugation, or N-linked and O-linked glycosylation; thus, certain modifications can significantly increase the weight of the protein fraction.
- Protein content on a weight basis in food or food ingredients can be defined in two ways: as the sum of the constituent amino acids, or as the sum of the constituent amino acids plus the weight of the prosthetic groups (for proteins that contain prosthetic groups).

Nitrogen is found in a variety of foods and food ingredients. Total nitrogen in foods comprises both protein nitrogen and non-protein nitrogen:

- The fraction of protein nitrogen is primarily defined by the sum of the nitrogen associated

with the amino acids that make up a protein. Proteins differ in their amino acid composition, and amino acids differ in their nitrogen content; hence, proteins differ in their total nitrogen content. In addition, a few of the prosthetic groups associated with proteins are nitrogenous (e.g. covalently linked coenzymes and amino sugars).

– Amino acids are not the only nitrogenous compounds in foods and food ingredients; other nitrogenous compounds include alkaloids, amines, ammonia, phospholipids and nitrogenous glycosides, nitrates, nitrites, nucleic acids, urea and vitamins. This non-protein nitrogen fraction varies greatly among food protein sources and in a given protein source, depending on the production process and the degree of purification of the protein source. For instance, non-protein nitrogen represents about 5% of the total nitrogen in cow's milk (Journet, Vérité & Vignon, 1975), between 7.1% and 9.3% in goat's milk (Grappin et al., 1981), and between 2.9% and 7.8% in defatted soybean flour (before heat treatment), in the form of free amino acids, nucleotides, creatine and choline (Maubois & Lorient, 2016).

– When describing a protein, free amino acids and small peptides are sometimes included and sometimes omitted; if they are included, they may be counted as part of either the protein nitrogen or the non-protein nitrogen.

The nitrogen content of individual amino acids varies according to the molecular weight of the amino acid and the number of nitrogen atoms it contains (from one to four, depending on the amino acid in question) (Sosulski & Imafidon, 1990). Thus, there are important differences in the nitrogen content of individual amino acids, distributed according to the amount of nitrogen in their residues:

– nitrogen-poor residues contain from 8.58% to 10.85% nitrogen: tyrosine, phenylalanine, methionine and glutamic acid;

– nitrogen-rich residues contain from 21.86% to 35.87% nitrogen: lysine, glutamine, asparagine, glycine, histidine and arginine; and

– the remaining 10 residues have a nitrogen content that ranges from 12.17% (aspartic acid) to 19.7% (alanine).

The variations in individual amino acid composition between proteins and in the nitrogen content of individual amino acids mean that the "generic" nitrogen content of protein is not 16%, but in reality can vary from about 13% to 19%. The fact that proteins from diverse sources vary in their nitrogen content owing to differences in their amino acid composition was first demonstrated through protein purification and nitrogen content analysis of extracted purified proteins (Jones, 1931).

2.2 General principles of NPCFs

For many years, the protein content of foods has been determined from total nitrogen content. The protein content in a food is traditionally estimated by multiplying the total nitrogen content by a NPCF, based on the assumptions that dietary carbohydrates and fats do not contain nitrogen, and that most of the nitrogen in the diet is present as amino acids in proteins. However, protein and nitrogen content are defined differently (Jones, 1931; Tkachuk, 1969; Sosulski & Imafidon, 1990; Mossé, 1990; Maubois & Lorient, 2016; Krul, 2019) (**Table 1**).

The conversion factor was historically set at 6.25 by assuming that most, if not all, nitrogen in food was derived from protein, and that the nitrogen content of proteins was about 16%. This

Table 1. The fractions of protein and nitrogen content in foods

PROTEIN CONTENT FRACTIONS	NITROGEN CONTENT FRACTIONS
Sum of weight of amino acid residues	Sum of weight of protein nitrogen from amino acid residues
Sum of weight of prosthetic groups	Sum of weight of non-protein nitrogen

approach is still an accepted method for calculating the crude protein content of foods and food ingredients; however, it has been known for decades that using total nitrogen content with a conversion factor of 6.25 to quantify total protein is imperfect and can lead to a 15–20% error in the actual protein content.

The errors for deriving the crude protein content from nitrogen content have three main origins, as outlined below:

- Total nitrogen content varies between the different amino acids; thus, because amino acid composition varies from one protein source to another, the protein nitrogen content also varies between different protein sources.
- In addition to amino acids, some proteins contain prosthetic groups, some of which are non-nitrogenous and therefore increase the molecular weight of the protein without significantly affecting the nitrogen content. In turn, this results in an increase in the conversion factor, because both amino acids and amino acids plus prosthetic groups are being considered on a weight basis.
- The fraction of non-protein nitrogen varies in different food protein sources; this can result in a lower value of the conversion factor when the total nitrogen content of foods is being considered.

The principle of NPCF calculation has been discussed in reviews and original articles (e.g. Jones, 1931; Heidelbaugh et al., 1975; Krul, 2019; Mariotti, Tome & Mirand, 2008; Maubois & Lorient, 2016; Mossé, 1990; Sosulski & Imafidon, 1990; Tkachuk, 1969). Because both the protein and the nitrogen component can be determined by different approaches, the resulting values of the conversion factor can vary. In the literature, different terminologies have been used for the conversion factors, depending on the method by which they have been calculated (e.g. K_A, K' and K_P).

2.3 Approaches for calculation of NPCFs

2.3.1 Crude protein weight versus amino acid composition

Initial approaches to calculating NPCFs involved extracting the crude protein fractions from the foods or food ingredients, determining the crude protein mass, measuring the nitrogen content in the isolated crude protein fraction, and then calculating a conversion factor from the nitrogen to protein ratio. This approach was used first for milk protein and later for additional protein sources (Hammersten, 1883; Jones, 1931). Although it provided a usable conversion factor, this early approach was not specific for the amino acids within a protein, because the crude protein mass included prosthetic groups when these were present in the protein; also, it did not explicitly account for any nitrogen from non-protein sources.

Newer approaches use values for protein weight and protein nitrogen content of different proteins based on knowledge of the amino acid composition as obtained via amino acid analysis or amino acid sequencing (Heathcote, 1950; Tkachuk, 1969; Holt & Sosulski, 1979;

Morr, 1981; Mossé, 1990). Such approaches have made it possible to derive more precise conversion factors, as discussed in **Section 3.2**. Calculation of the weight of the protein can include either the sum of the amino acid residues only, or the sum of the amino acid residues plus the weight of the associated prosthetic groups when these are analysed by specific methods or obtained from amino acid sequencing.

2.3.2 Anhydrous weight of amino acids

An important feature of approaches based on amino acid composition is the use of the anhydrous weight of amino acids to determine protein weight. Using the free amino acid weight for a protein would grossly overestimate the weight for longer polypeptide chains. Because each amino acid residue in a polypeptide chain loses one water molecule (weighing 18 Da) during polymerization, except for the residue at the carboxy terminus; thus, the weight of the protein must be assessed as the sum of the weight of the *anhydrous* amino acid residues rather than the weight of the *free* amino acids (each of which weighs 18 Da more than its anhydrous counterpart). Molecular weights of free and anhydrous forms of all 20 amino acids, and the corresponding percentage of nitrogen, are shown in **Table 2**.

2.3.3 Contribution of amide amino acids to protein nitrogen content

Another important consideration when calculating NPCFs relates to the differential contribution to nitrogen content of the amide forms of the amino acids, glutamine and asparagine, and their respective acid forms, glutamic acid and aspartic acid.

With standard amino acid analysis of a protein sample, glutamine and asparagine are converted to glutamic acid and aspartic acid during acid hydrolysis (by substitution of a carboxyl group for the amide group); the total of glutamic acid plus glutamine and aspartic acid plus asparagine is then determined. This conversion does not significantly affect the calculation of the total anhydrous amino acid weight, because there is little difference between the molecular weights of glutamic acid and glutamine (147.13 Da and 146.15 Da, respectively) and aspartic acid and asparagine (133.11 Da and 132.12 Da, respectively). However, failing to consider the ratio between the amide and acid forms induces a major error in the calculation of total nitrogen content of the amino acid residues.

The nitrogen content of the amide amino acids can be determined using specific analytical conditions that help to preserve the amide nitrogen, although the different conditions have drawbacks in terms of accuracy in estimating the true nitrogen content. For example, using mild acid hydrolysis conditions (e.g. 2 M hydrochloric acid [HCl], 3 hours, 115 °C) and measuring the released ammonia (NH3) allows for the quantification of amide nitrogen, but it does not differentiate between nitrogen from asparagine and from glutamine (Mossé, 1990). Alternatively, the total NH3 released from asparagine and glutamine during acid hydrolysis with 6 N HCl can be used as a proxy for amide nitrogen content (Heathcote, 1950; Tkachuk, 1969), recognizing that this value is equal or slightly higher than the true amount of amide NH3, leading to an overestimation of nitrogen from the amide form of amino acids. This latter approach includes nitrogen released from amide asparagine and glutamine, but also from other amino acids (serine and threonine) and from other non-protein sources of nitrogen. Yet another approach is to consider a fixed ratio between acid and amide forms that is specific for each protein source (often 75/25 or 50/50), as found in the literature. Specific derivatives can also be used to determine asparagine and glutamine directly.

Table 2. Values used to calculate NPCFs

Amino acid	Molecular weight (Da)[a]		AA residue/ free AA (%)	N in free AA (%)	N in AA residue (%)
	Free AA	AA residue			
Aspartic acid	133.1	115.1	86.5	10.5	12.2
Threonine	119.1	101.1	84.9	11.8	13.9
Serine	105.1	87.1	82.9	13.3	16.1
Glutamic acid	147.1	129.1	87.8	9.5	10.8
Proline	115.1	97.1	84.4	12.2	14.4
Glycine	75.1	57.1	76.0	18.7	24.6
Alanine	89.1	71.1	79.8	15.7	19.7
Cysteine	121.2	103.1	85.1	11.6	13.6
Valine	117.1	99.1	84.6	12.0	14.1
Methionine	149.2	131.2	87.9	9.4	10.7
Isoleucine	131.2	113.2	86.3	10.7	12.4
Leucine	131.2	113.2	86.3	10.7	12.4
Tyrosine	181.2	163.2	90.1	7.7	8.6
Phenylalanine	165.2	147.2	89.1	8.5	9.5
Tryptophan	204.2	186.2	91.2	13.7	15.0
Lysine	146.2	128.2	87.7	19.2	21.9
Histidine	155.2	137.1	88.4	27.1	30.6
Arginine	174.2	156.2	89.7	32.2	35.9
Asparagine	132.1	114.1	86.4	21.2	24.6
Glutamine	146.1	128.1	87.7	19.2	21.9
Ammonia	17	16	94.1	82.2	87.4
Water	18	–	–	–	–

AA: amino acid; Da Dalton; N: nitrogen; NPCF: nitrogen to protein conversion factor.

[a] The weight of each AA residue is the weight of the free AA minus 18 Da (to account for the loss of one water molecule).

Sources: Sosulski and Imafidon (1990) and Laurens, Olstad and Templeton (2018).

3. METHODS

3.1 Systematic review of the literature

This review was conducted following the JEMNU terms of reference and rules of procedure (FAO/WHO, 2012), including the development of key questions to guide the systematic review in PICO (population, intervention, comparator and outcome) format, and assessment of the certainty in the evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework.[1]

3.1.1 PICO questions

The purpose of this review is to provide the background for the identification of appropriate NPCFs for soy-based and milk-based ingredients used in infant formula and follow-up formula.

The nature of the desired information does not lend itself easily to the PICO format, because strictly speaking we are not dealing with populations, interventions, comparators or different health outcomes. Nevertheless, the key questions needed to guide this review were adapted to PICO format as far as possible and, in keeping with the intent of PICO questions, were framed to be as specific as possible for the problem at hand and to facilitate the search for relevant answers.

The original PICO questions were formulated as follows:

1. When determining the protein content of soy-based ingredients used in infant formula and follow-up formula, what is the appropriate science-based NPCF to use when comparing protein content derived from nitrogen-based methods to amino acid-based methods?
2. When determining the protein content of milk-based ingredients used in infant formula and follow-up formula, what is the appropriate science-based NPCF to use when comparing protein content derived from nitrogen-based methods to amino acid-based methods?

These questions were further refined to sets of PICO questions, with different assumptions regarding the definition of protein:

1. When using the equation *amount of protein (P) = NPCF (K) * amount of nitrogen (N)* to estimate the protein content of dairy-based ingredients used in infant formula and follow-up formula, which value of K most closely estimates the true amount of protein (P), where "protein" is defined as amino acid content only?
2. When using the equation *amount of protein (P) = NPCF (K) * amount of nitrogen (N)* to estimate the protein content of soy-based ingredients used in infant formula and follow-up formula, which value of K most closely estimates the true amount of protein (P), where "protein" is defined as amino acid content only?

and

3. When using the equation *amount of protein (P) = NPCF (K) * amount of nitrogen (N)* to estimate the protein content of dairy-based ingredients used in infant formula and follow-up formula, which value of K most closely estimates the true amount of protein (P), where "protein" is defined as amino acid plus prosthetic groups?

[1] See http://www.gradeworkinggroup.org/.

4. When using the equation *amount of protein (P) = NPCF (K) * amount of nitrogen (N)* to estimate the protein content of soy-based ingredients used in infant formula and follow-up formula, which value of K most closely estimates the true amount of protein (P), where "protein" is defined as amino acid plus prosthetic groups?

In the revised PICO questions, the population is considered to be infant formula and follow-up formula, and the outcome different conversion factors. Although different values are being compared, there is no direct comparator as part of an intervention. In each case, "nitrogen (N)" may be total nitrogen or total nitrogen corrected for non-protein nitrogen (i.e. protein nitrogen).

3.1.2 Literature review

3.1.2.1 Databases and search terms

The following databases were searched on March 2019, from 1946 to the present:

- Web of Science Core Collection – Clarivate Analytics (using the field "Topic");
- Medline/PubMed of the National Library of Medicine (using the field "All fields");
- CAB Direct/CAB Abstracts – CABI (using the field "All fields"); and
- Food Science and Technology Abstracts (FSTA) – Ebsco/IFIS (using the field "All fields").

The search terms used are provided in **Annex 1**. In addition to the primary search on NPCFs, a secondary search was conducted to summarize methods for nitrogen and protein analysis, to provide background for a discussion of the results of the review on conversion factors. Reference lists of identified papers were hand searched for additional relevant citations.

3.1.2.2 Call for data

Data on NPCFs are often generated but not published, meaning that unpublished data might be an important source of information; FAO and WHO therefore issued a call for data in November 2018.[1] The call was disseminated through the FAO and WHO websites, the United Nations System Standing Committee on Nutrition (UNSCN) network, and the Codex Alimentarius network.

3.1.3 Data analysis

NPCFs were extracted from published literature and from unpublished reports, where available. Where conversion factors were not reported, but data were available on protein, nitrogen and amino acid analysis, these data were used to calculate the conversion factor(s) (**Annex 2**). Similarly, NPCFs were recalculated for data in which a conversion factor was reported but either the method of calculation was unclear, or the data provided made it impossible to check the values obtained.

The mean and 95% confidence interval (CI) were calculated for data that were pooled by class of conversion factor across all dairy-based foods, and separately for all soy-based foods. The conversion factor classes, described below, are K′, K_B and K_A 50/50, K_A 75/25, and K_A direct adjustment.[2] In addition, dispersion of measurements within different types of

[1] At https://www.who.int/nutrition/topics/datacall-nitrogenprotein-2018/en/ (no longer posted on FAO website).

[2] In these conversion factor classes, 50/50, 75/25 and "direct adjustment" refer to the ratios of amide to glutamic and aspartic acid residues used in the calculation for K_A.

foods (e.g. cow's milk, milk-based infant formula, soybean and soy isolate) was assessed by standard boxplot analysis.

3.2 Calculation of NPCFs

In this review, conversion factors are designated by K′, KA or KB, depending on how they were calculated.

The conversion factor KA considers the crude protein solely in terms of amino acid residues (Heidelbaugh et al., 1975; Tkachuk, 1969; Sosulski & Imafidon, 1990; Mossé, 1990; Morr, 1981; 1982). It is calculated as the ratio of the sum (∑) of the weight of anhydrous amino acids (determined by amino acid analysis) to protein nitrogen, which is calculated by summating only the nitrogen content of the amino acid residues found in the sample. The equation for calculating K_A is thus:

$$K_A = \sum \text{anhydrous amino acids residues} / PN$$

where "PN" is protein nitrogen.

Except for some highly purified protein fractions, most samples contain non-protein nitrogen (i.e. nitrogen from sources other than protein). K_A will therefore tend to overestimate the amount of protein in samples with appreciable amounts of non-protein nitrogen, because it considers only nitrogen from protein. Such overestimation will only occur if the factor K_A is used to multiply total nitrogen. If total nitrogen is corrected for non-protein nitrogen following its analysis along with total nitrogen, then the factor leads to accurate values. Thus, K_A can provide accurate values in samples with an appreciable amount of non-protein nitrogen, provided that the non-protein nitrogen is measured and subtracted from the total nitrogen measurement, yielding protein nitrogen.

K_B takes non-protein nitrogen into consideration and is calculated as the ratio of the sum of the weight of all anhydrous amino acids residues to total nitrogen content, which includes both protein nitrogen and non-protein nitrogen (Holt & Sosulski, 1979; Mossé, 1990). The equation for calculating K_B is thus:

$$K_B = \sum \text{anhydrous amino acids residues} / (PN+NPN)$$

where "NPN" is non-protein nitrogen.

Because K_B considers the presence of non-protein nitrogen in the sample, it has been called the "corrected" conversion factor. It is not necessary to adjust for the amide to acid ratio when calculating KB (or K′ with total nitrogen content), because the entire amount of nitrogen is measured, and thus the actual amount of nitrogen supplied by amide and acid forms of amino acids is already included. However, this factor can lead to inaccuracies because the non-protein nitrogen fraction can vary across different samples for a particular class of protein.

In contrast to K_A and K_B, which only consider the weight of the anhydrous amino acids, the conversion factor K′ includes the total weight of the protein, and is calculated as the ratio of the sum of the weights of anhydrous amino acids plus the sum of the weights of characterized prosthetic groups obtained from complete amino acid analysis of the protein, to either protein nitrogen or total nitrogen (Van Boekel & Dumas, 1987; Rouch et al., 2008; Maubois & Lorient, 2016). The equation for calculating K′ is thus:

$$K' = (\sum \text{anhydrous amino acids residues} + \sum \text{prosthetic groups}) / (PN \text{ or } PN+NPN)$$

K′ therefore provides a conversion factor that is close to the early factors obtained in the first calculations used for determining protein concentrations. In those calculations, the crude protein was purified and its weight was measured, but without knowledge of the amino acid composition or prosthetic groups (Hammersten, 1883; Jones, 1931).

There are few K′ values based on protein weights that include prosthetic groups that have been fully characterized and accounted for by amino acid analysis; those that are available are generally limited to dairy proteins and dairy-based ingredients. Because prosthetic groups can add considerably to the weight of proteins, K′ will overestimate the amino acid content of the sample when prosthetic groups are present. In addition, the prosthetic groups are susceptible to processing conditions and are therefore likely to vary both within and among protein sources. To be meaningful, the prosthetic groups should be defined based on the protein post-processing.

K′ is related to K_A and K_B via the following equations:

$$K' = K_A + (\sum \text{prosthetic groups} / PN)$$

$$K' = K_B + (\sum \text{prosthetic groups} / PN+NPN)$$

Further, because K′ includes prosthetic groups when measuring the weight of protein, and KB includes non-protein nitrogen when assessing nitrogen content, generally:

$$K' \geq K_A \geq K_B$$

For a given sample containing prosthetic groups and non-protein nitrogen:

$$K' > K_A > K_B$$

For a given sample without prosthetic groups or non-protein nitrogen:

$$K' = K_A = K_B$$

3.3 Modelling of non-protein nitrogen

KB values were modelled for different amounts of theoretical non-protein nitrogen from the different published values of K_A with amide to acid ratios that were directly assessed and adjusted, or were set arbitrarily at 50/50 or 75/25, as listed in in **Table 12** and **Table 13**.

Missing data were imputed by taking the median for a particular set of KA data. For example, for cow's milk, in column K_A – subcolumn (a) of **Table 13** – the third, fourth and fifth missing values (corresponding to Sosulski and Imafidon (1990) and Fujihara, Sasaki and Sugahara (2010), respectively) were replaced with the median of the series, 6.09. The use of the statistical median (rather than the mean or other statistical quantity) avoids working with sparse matrices and guarantees a certain robustness of the modelling.

The data were modelled using the following equation and parameters:

$$K_B = K_C * \frac{PN}{[PN + (TN * \%NPN)]}$$

where:

K_B = conversion factor taking into account non-protein nitrogen
K_A = conversion factor assuming 0% non-protein nitrogen
TN = total nitrogen content
PN = Protein content
NPN = non-protein nitrogen

and where total nitrogen and protein nitrogen content values for soy products and dairy products come from Boisen, Bech-Andersen and Eggum (1987) and Journet et al. (1975), respectively, as shown in **Table 3**.

Table 3. Total nitrogen and protein nitrogen content values for soy-based products and dairy-based products

(g/kg of dry product)	Soy-based products	Dairy-based products
Protein nitrogen	67	62
Total nitrogen	69	78

Sources: Boisen et al. (1987) and Journet et al. (1975).

3.4 Assessment of risk of bias and certainty of evidence

Cochrane criteria were used to assess risk of bias for each study where applicable, including selective reporting and measurement error (The Cochrane Collaboration, 2011). Some of the Cochrane criteria were not relevant because of the analytical nature of the included studies. The certainty of the evidence was assessed using GRADE, and was modified as necessary to accommodate the unique nature of the included studies, which were not epidemiological or otherwise intervention-based.

Although the studies were analytical in nature and used well-established, objective methods for assessing amino acid composition and nitrogen analysis, measurements made with these methods have inherent inaccuracy. Therefore, conversion factors derived primarily from these types of methods started at "moderate" certainty in the evidence and were downgraded as necessary, based on judgements regarding risk of bias, inconsistency, indirectness and imprecision. Conversion factors derived primarily from studies in which the more accurate method of amino acid sequencing was used started at "high" certainty in the evidence.

Owing to the highly analytical nature of the studies, and the inability to establish a threshold value for treatment, benefit or harm, imprecision was assessed based on the number of values reported only, with a minimum of 10 values required to prevent downgrading once for serious imprecision, and a minimum of five studies to prevent downgrading twice for very serious imprecision. Inconsistency was assessed via the 95% confidence interval (CI) of the mean, with variation around the mean of more than 10% considered to be serious inconsistency.

4. RESULTS

4.1 Literature review

Regarding the search for NPCFs, after removing duplicates, a total of 3881 publications were initially identified. These publications were mainly scientific articles, but they also included 34 books, 27 book chapters, one report, 12 theses and 23 patents (from CAB Direct and FSTA).

Titles and keywords of these publications were screened for exclusion of background noise articles, followed by further screening of abstracts and article contents, and additional analysis of the bibliography of the selected articles; this resulted in 67 publications being included in the analyses for NPCFs (**Fig. 2**). Reasons for exclusion of studies included reports of previously published data, publications using the conversion factor of 6.25 or another fixed value but not addressing the calculation, articles addressing products other than milk and soy or not relevant to the objective of the review, and some articles already cited and not providing additional information (**Annex 3**).

The 67 articles selected for analysis of NPCFs were published between 1883 and 2019 (some historical papers were identified through reference lists), and they included 63 articles in scientific journals, one article in a proceedings book, one report from a public organization (United States Department of Agriculture), one report from the American Oil Chemists Society (AOCS) and one report from the dairy industry. The scientific journals were related to dairy science (12 articles), food science (27 articles), plant and agricultural sciences (15 articles) and other related disciplines (e.g. nutrition, physiology, chemistry and biochemistry); they included four reviews and 58 articles with original data. Of the 63 articles from scientific publications, 61 were from academic institutions and about 30% declared a funding contribution from industry or a private organization. The studies were conducted in Australia, China, Europe, New Zealand and North America.

In addition to the studies identified in the literature review, a total of eight submissions were received from the public call for data, five of which were included in the analyses.[1] Three sets of data were reported in other published or non-published documents already cited in the review, or did not provide data on nitrogen and/or amino acid analysis, and were therefore not included. These sets of data were:

- Agri-Food Canada: reference already cited (Zarkadas et al., 2007);
- Fonterra: amino acid analysis not provided; and
- International Dairy Federation (IDF): data already included in (Maubois & Lorient, 2016).

A separate search was conducted to identify publications relevant for a narrative summary of methods for nitrogen and protein analysis.[2] The searches identified 476 relevant publications, of which 215 were used to inform the summary. These 215 articles are included in the bibliography for information, but not all of these articles were analysed in detail. Results of this search are reported as part of the background in **Section 4.2**.

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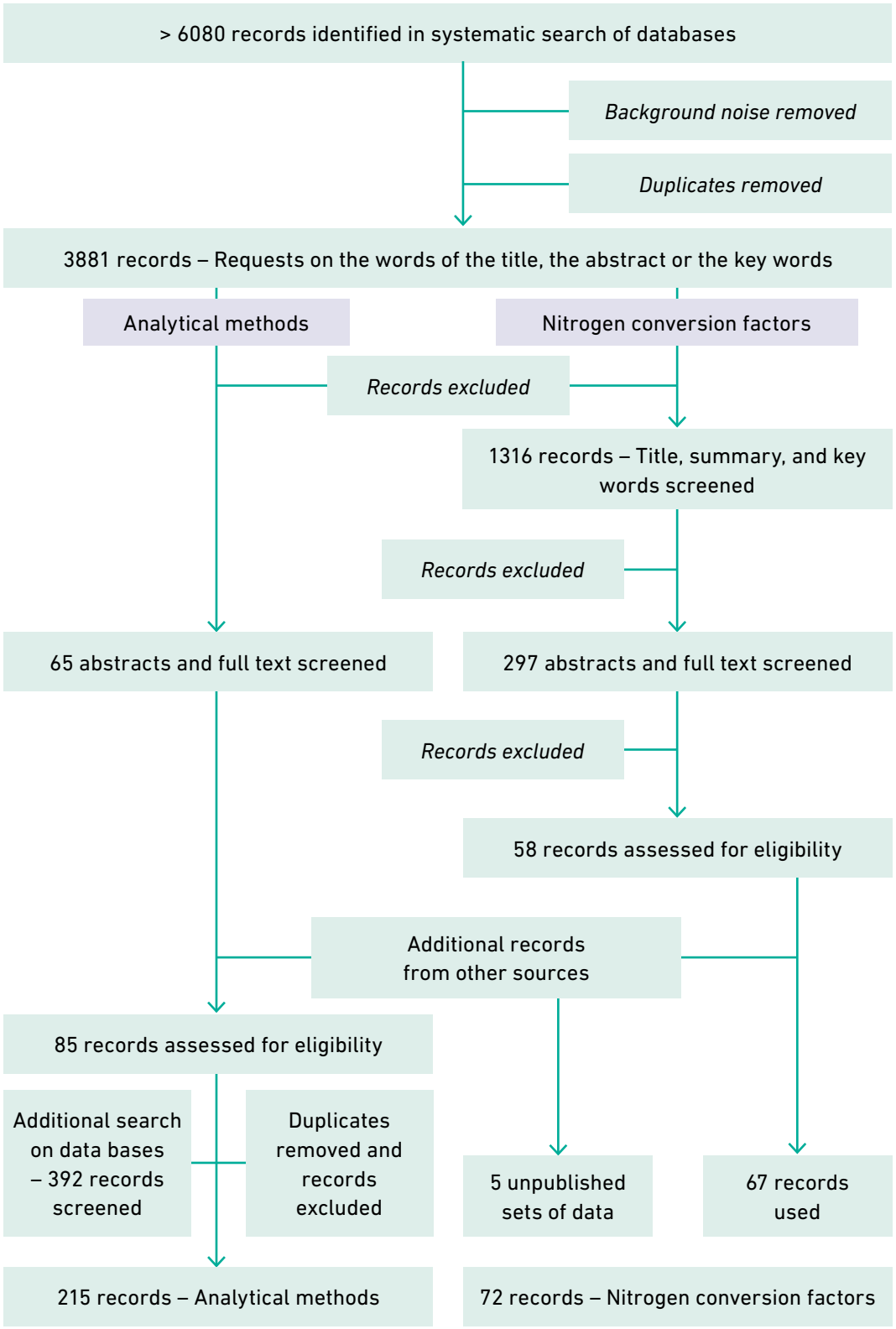
[1] Submissions were received from the following: Japan Vegetable Food Association (milk and soy), Nestec (milk and soy), Dupont (soy), Nutrition Research Division – Bureau of Nutritional Sciences – Health Canada (soy), Agri-Food Canada (soy), ENSA (soy), Fonterra (whey products), and International Dairy Federation (milk, soy).

[2] The search was conducted from within the 3881 articles identified in the main search, plus publications in the period 1956–2019 compiled from the ISI Web of Knowledge or other sources (Google Scholar, Science Direct, Wiley) with the words "analytical methods", "Kjeldahl" and "Dumas method" in the title.

Fig. 2 PRISMA flow diagram of study selection for analytical methods and NPCFs



NPCF: nitrogen to protein conversion factors; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

4.2 Analytical methods for nitrogen, amino acid and protein content in foods

Methods for analysis of food proteins are characterized according to their analysis of total nitrogen, amino acid content and total protein content (**Table 4**). Other common assays include various dye-binding methods that are less suited for routine analysis. Some key references are Leca-Bouvier and Blum (2005); Moore et al. (2010); Noble and Bailey (2009); Owusu-Apenten (2002); Sapan and Lundblad (2015); Simonian and Smith (2006); (Simonian, 2002); Wallace and Fox (1998).

Table 4. Methods for food protein analysis

TECHNIQUE	REFERENCE
NITROGEN ANALYSIS	
Kjeldahl method	Kjeldahl (1883)
Dumas method	(Dumas, 1831)
AMINO ACID ANALYSIS	Moore and Stein (1948; 1954)
PROTEIN ANALYSIS	
Biuret, Lowry, Bradford, Coomassie blue dye – binding, bicinchoninic acid	Gornall, Bardawill and David (1949); Lowry et al. (1951); Peterson (1979); Legler et al. (1985); Bradford (1976); Compton and Jones (1985)
Nessler reagent	Wanklin and Chapman (1874)
Berthelot's method (alkali-phenol reagent)	Berthelot (1859)
Folin-Ciocalteu method	Folin and Ciocalteu (1927)
Dye binding	Fraenkel-Conrat and Cooper (1944)
Direct alkaline distillation	Owusu-Apenten (2002)
Modified Berthelot reaction	Searle (1984)
3-(4-Carboxybenzyl)quinoline-2-carboxaldehyde	You et al. (1997)
Near infrared reflectance (NIR)	Owusu-Apenten (2002)

Source: adapted from Sáez-Plaza et al. (2013a).

4.2.1 Methods to determine total nitrogen content in foods

The Kjeldahl and Dumas methods, described below, are examples of methods used to determine nitrogen content.

4.2.1.1 Kjeldahl method

The Kjeldahl method was originally designed for the brewing industry, for monitoring protein changes in grain during germination and fermentation (Kjeldahl, 1883; Bradstreet, 1954). First published in 1883, the method has been accepted with modifications as the standard for the determination of nitrogen content (Lynch, Barbano & Fleming, 1998; Moore et al., 2010; Kjeldahl, 1883; Bradstreet, 1954) (**Table 5**). The Kjeldahl method and devices have been significantly modified over the past 100 years, but the basic principles are still valid and include three main steps (Adesina, 2012; Benton Jones Jr., 1991):

Table 5. Kjeldahl methods and modifications for total protein measurement in food, by matrix

SPECIFIC MATRIX	METHOD REFERENCE	SPECIFIC MATRIX	METHOD REFERENCE
Cereal grains	AOAC 979.09; AACC 46–11A; AACC 46–16; AACC 46–16; (Beljkaš et al., 2010; Salo-väänänen & Koivistoinen, 1996)	**Fruit products**	AOAC 920.152
Cereal adjuncts	AOAC 945.18; AACC 46–11A; AACC 46–16; (Gouveia et al., 2014)	**Gelatin**	AOAC 935.46
Flour (wheat)	AOAC 920.87; AACC 46–11A; AACC 46–12; AACC 46–13; AACC 46–16; AACC 46–30; (Ronalds, O'Brien & Allen, 1984)	**Infant formula (milk-based)**	AOAC 986.25 (Brooke, Wood & Barley, 1982)
Soy flour	AOCS Bc 4–91	**Laboratory malt**	AOAC 950.09
Soybeans	AOCS Ac 4–91	**Maccaroni products**	AOAC 930.25
Meat	AOAC 981.10	**Milk chocolate**	AOAC 939.02
Cow's milk (fluid) (whole, partially skimmed or skimmed milk)	AOAC 991.20; ISO-IDF 8968–1/20–1:2001; ISO-IDF 8968–2/20–2:2001; ISO-IDF 8968–3/20–3:2004 ; ISO 8968–1:2014 and IDF 20–1:2014	**Nuts and nut products**	AOAC 950.48
Dried cow's milk and milk products[a]	AOAC 930.29 ; ISO 8968–1:2014 and IDF 20–1:2014	**Peanuts**	AOCS Ab 4–91
Cream	AOAC 920.109	**Plants**	AOAC 977.02; AOAC 978.04
Baked products	AOAC 935.39	**Starch dessert powder**	AOAC945.56
Beer	AOAC 920.53	**Sunflower seeds**	AOCS Ai 4–91
Bread	AOAC 950.36	**Sweetened condensed milk**	AOAC 920.115
Brewing sugars and syrups	AOAC 945.23	**Tea**	AOAC 920.103
Cacao	AOAC 970.22	**Wine**	AOAC 920.70
Caseins and caseinates	ISO 5549:1978; ISO 8968–1:2014 and IDF 20–1:2014	**Yeast**	AOAC 962.10; AACC 46–11A; AACC 46–16
Cheese (hard, semi-hard and processed cheese)	AOAC 920.123; AOAC 2001.14; ISO 8968–1:2014 and IDF 20–1:2014	**Goat's and sheep's milk (whole milk)**	ISO 8968–1:2014 and IDF 20–1:2014

AACC: American Association of Cereal Chemists; AOAC: AOAC International; AOCS: American Oil Chemist's Society; IDF: International Dairy Federation; ISO: International Organization for Standardization.

[a] Including milk-based infant formula, milk protein concentrate, whey protein concentrate, casein and caseinate.

1. *Digestion* – the decomposition of nitrogen from organic samples by boiling in concentrated sulfuric acid resulting in an ammonium sulfate solution.
2. *Distillation* – adding excess base to the acid digestion mixture, which converts methane (NH_4+) to ammonia (NH_3), followed by boiling and condensation of the NH3 gas in a receiving solution.
3. *Titration* – the amount of ammonia in the receiving solution is quantified.

This method has been discussed extensively in various reviews (e.g. Dyer, 1895; Morries, 1983; Benton Jones Jr., 1987; Horneck & Miller, 1998; Lynch & Barbano, 1999; Owusu-Apenten, 2002; Moore et al., 2010; Sáez-Plaza et al., 2013a; Sáez-Plaza et al., 2013b; Sapan & Lundblad, 2015).

The Kjeldahl method provides total nitrogen content; it does not distinguish between protein-based nitrogen and non-protein nitrogen, including inorganic nitrogen and other organic nitrogen (e.g. urea, melamine, nucleotides and nucleic acids). Inorganic nitrogen is often negligible in food and biological samples (Simonne et al., 1997), but urea may not be negligible, depending on the type of biological samples analysed (Grappin, 1992). The nitrogen-containing nitrate portion can be higher in products from vegetables fertilized with nitrate-based fertilizers, and the recovery rate during the Kjeldahl digestion procedure affects the determination of total nitrogen (Simonne et al., 1994). The limitations of the method have been studied extensively, and many improvements have been made to each of the steps in the process (Watson & Galliher, 2001).

4.2.1.2 Dumas method

As with the Kjeldahl method, the Dumas method is considered a direct method for determination of total nitrogen content. It was developed in 1831 by Dumas, from the observation that nitrogenous compounds heated with alkali give ammonia, which can be determined volumetrically (Szabadváry & Robinson, 1980). The method was further improved by igniting the sample with barium hydroxide, and quantifying the ammonia liberated into HCl gravimetrically by precipitation as ammonium hexachloroplatinate (Szabadváry & Robinson, 1980). In a modified method, the ammonia is absorbed in a known amount of HCl, and the excess is back-titrated using lime dissolved in water containing sugar (Klosterman, 1985). This process can be fully automated, taking only a few minutes per measurement (Saint-Denis & Goupy, 2004; Whitesell et al., 2014).

Currently, the method consists of combusting a sample of known mass in a high-temperature (range, 800–900 °C) chamber in the presence of oxygen, producing carbon dioxide (CO_2), water and nitrogen. The gases are then passed over special columns (e.g. those containing a potassium hydroxide aqueous solution) that absorb CO_2 and water. A column containing a thermal conductivity detector at the end is then used to separate the nitrogen from residual CO_2 and water, and the remaining nitrogen content is measured. The instrument must initially be calibrated by analysing a pure material containing a known nitrogen concentration. The measured signal from the thermal conductivity detector for the unknown sample is converted into a nitrogen content.

As with the Kjeldahl method, the Dumas method does not give a measure of true protein because it includes non-protein nitrogen, and different correction factors are needed for each protein because each has a different amino acid sequence. Total nitrogen values calculated by the Dumas method can be slightly higher than the Kjeldahl values because Dumas nitrogen also includes nitrates and organic compounds that are highly resistant to acid digestion (Lakin, 1978; Petterson et al., 1999). As a result, differences can occur between the two methods with some types of food (e.g. fruits, vegetables and fish).

A Kjeldahl nitrogen and Dumas nitrogen correction factor of 0.15 has been proposed (Simonne et al., 1997). During harvest years 2000–2004, the Max Rubner Institute in Detmold, Germany, performed a comprehensive study of more than 800 wheat samples, comparing the crude protein results between the Kjeldahl and Dumas methods. The study found that some 2% of "Dumas protein" was not determined by the Kjeldahl method, and presented the following relationship between Dumas and Kjeldahl protein values: Kjeldahl = 0.959 * Dumas + 0.258 (Müller, 2017). However, this equation cannot be generalized to other plant and food sources, because different factors (e.g. cultivar, growing year and conditions) can modify the results.

A relationship was also proposed for plant tissues in general: Kjeldahl = 0.85 * Dumas + 0.0.015 (Simonne et al., 1994). In some selected food groups, the Dumas method can replace the Kjeldahl method by using correction factors (Simonne et al., 1997); however, the differences between the two methods are often not statistically significant (Mihaljev et al., 2015). Some of the differences between the two methods when applied to the same sample can also depend on the type of catalyst used, and this may be a further source of variability.

The number of publications related to the Dumas method is much lower than for the Kjeldahl method; however, when the use of mercury and cadmium in the laboratory was banned in most countries in the 1990s, the Dumas method was evaluated as an alternative to the Kjeldahl method (**Table 6**). The Dumas method has been automated (Leco analyser) and is now routinely used in many laboratories.

Table 6. Standards for total nitrogen and protein measurement in food and feed using the Dumas method

REFERENCE	TITLE (MATRIX)
ISO 16634–1:2008	Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content – Part 1: Oilseeds and animal feeding stuff.
ISO 16634–2:2016	Food products – Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content – Part 2: Cereals, pulses and milled cereal products.
ISO 14891:2008 (IDF 185:2008)	Milk and milk products — Determination of nitrogen content — Routine method using combustion according to the Dumas principle.
AACC Method 46.30	Crude protein — Combustion method (animal feeds, cereals and oil seeds).
ICC Standard No. 167	Determination of crude protein in grain and grain products for food and feed by the Dumas combustion principle.
AOAC 990.03	Protein (crude) in animal feed — Combustion method.
AOAC 992.23	Crude protein in cereal grains and oil seeds.
AOAC 997.09	Nitrogen in beer, wort, and brewing grains — Protein (total) by calculation — Combustion method.
AOCS Ba 4e-93	Generic combustion method for determination of crude protein (oilseed byproducts).
AOCS Ba 4f-00	Combustion method for determination of crude protein in soybean meal.
OIV-MA-AS323–02A	Quantification of total nitrogen according to the Dumas method in musts and wines (Type II method).

AACC: American Association of Cereal Chemists; AOAC: AOAC International; AOCS: American Oil Chemists' Society; ICC: International Association for Cereal Science and Technology; IDF: International Dairy Federation; ISO: International Organization for Standardization; OIV: International Organization of Vine and Wine.

4.2.2 Methods for amino acid analysis

Amino acid analysis was first developed around 1950 (Moore & Stein, 1948; 1954) to determine the amino acid content of pure proteins. Before performing such analysis, it is necessary to hydrolyse a protein to its individual amino acid constituents. The amino acid constituents of the test sample are typically derivatized for analysis. The techniques commonly used to separate free amino acids are ion-exchange chromatography, gas chromatography, high-performance liquid chromatography (HPLC) and its ultra-fast variant UHPLC, and electrophoresis.

4.2.2.1 Sample hydrolysis

Hydrolysis of protein and peptide samples is necessary for amino acid analysis. Hydrolysis with HCl is performed at 110 °C for 20–70 hours, with the temperature being accurately controlled. After hydrolysis, residual HCl is removed in a rotary evaporator, and the residue is dissolved in water or buffer, depending on whether the separation method will be ion-exchange chromatography or HPLC. The standard method of hydrolysis is incubation in an oxygen-free environment in constant boiling 6 M HCl at 110 °C for 18–24 hr, with 24-hour hydrolysis being used most often (Ozols, 1990; Darragh & Moughan, 2005). The acid hydrolysis releases free amino acids but can produce uncontrolled complete or partial destruction of several amino acids (**Table 7**). Owing to the unstable behaviour of amino acids under the hydrolysis conditions described above, of the 20 amino acids commonly present in proteins, only 10 can be determined quantitatively after acid hydrolysis; they are aspartic acid, glutamic acid, proline, glycine, alanine, leucine, phenylalanine, histidine, arginine and hydroxyproline. Amino acids that are totally or partially destroyed include tryptophan, cysteine, serine and threonine, while methionine might undergo oxidation. In peptide bonds involving isoleucine and valine, the amino bonds of isoleucine–isoleucine, valine–valine, isoleucine–valine and valine–isoleucine are partially cleaved. The amide amino acids asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively.

Not all amino acids are fully released or are partially destroyed under conditions of standard hydrolysis (i.e. 24-hour acid hydrolysis); hence, values obtained with this method can only be considered as approximations. More accurate information on amino acid content of a protein can be obtained by using multiple hydrolysis times and fitting data to a curvilinear model that gives instantaneous rates of release and destruction, and that can be used to accurately predict the amino acid composition of the protein (Rutherfurd & Moughan, 2018). The model can be applied to acid hydrolysis, acid hydrolysis after performic acid oxidation for sulfur amino acids, and alkaline hydrolysis for tryptophan.

Several hydrolysis techniques have been described and used to address these concerns. For example, formation of cysteic acid, methionine sulfoxide and chlorotyrosine can be avoided if all air is removed from the sample before hydrolysis. The HCl hydrolysis procedure also minimizes decomposition of reduced S-carboxymethylcysteine and preserves S-carboxymethylated amino acids. To overcome problems associated with the substantial loss of amino acid residues during acid or basic hydrolysis, enzymatic hydrolysis can be used to hydrolyse proteins from animal tissues or foods to obtain amino acids in soluble form for optimizing the recovery of glutamine, glutamate, asparagine and aspartate residues (Peace & Gilani, 2005; Tsao & Otter, 1999). The use of enzymatic hydrolysis is interesting compared with acid or basic hydrolysis, because it avoids the racemization of certain amino acid residues (e.g. 2–5% for aspartate, histidine, lysine and methionine residues with conventional methods and with microwave heating in the absence of phenol). Weiss et al. (1998) provided a list of 45 protein hydrolysis methods with their corresponding references.

4.2.2.2 Chromatography

There are several chromatographic techniques for amino acid analysis, and the choice depends on the sensitivity required for the assay. The separation of free amino acids by ion-exchange chromatography followed by post-column derivatization with ninhydrin is the traditional amino acid analysis technique (Moore & Stein, 1948). Post-column detection techniques can be used with samples that contain small amounts of buffer components (e.g. salts and urea), and generally requires 5–10 µg of protein sample per analysis.

Table 7. Problematic amino acids during the protein hydrolysis procedure

AMINO ACID	DRAWBACK DURING HYDROLYSIS	POSSIBLE SOLUTIONS	REFERENCES
Tryptophan	Destroyed in presence of carbohydrates, starch or sugars and heavy metals	• Add thiols • Use alkaline hydrolysis: sodium hydroxide, lithium hydroxide, barium di-hydroxide[a] • Use Teflon or polypropylene containers to reduce the presence of heavy metals	Finley (1985); Hanko and Rohrer (2002); Landry, Delhaye and Jones (1992); Rutherfurd and Gilani (2009); Steinhart (1984); Rowan, Moughan and Wilson (1989); Bech-Andersen (1991)
Cysteine and methionine	Cysteine is destroyed, methionine is oxidized to methionine sulfoxide and methionine sulfone	• Remove oxygen from the hydrolysis tube • Use method with performic acid • Use methods that do not involve oxidation including reaction with cyanogen bromide • Use methods with methanesulfonic acid, p-toluenesulfonic acid or alkaline hydrolysis	Rutherfurd and Gilani (2009); Ellinger and Duncan (1976); Elias, McClements and Decker (2005); Hayashi and Suzuki (1985); Sochaski et al. (2001); Cuq et al. (1978); Todd, Marable and Kehrberg (1984)
Asparagine and glutamine	Deamination to produce aspartic acid and glutamic acid leading to an overestimation of aspartic acid and glutamic acid	• Esterification-reduction • Carbodiimide modification • Enzymatic hydrolysis • Amide to amine conversion[b]	Wilcox (1967); Carraway and Koshland (1972); Soby and Johnson (1981)
Serine and threonine	Partially destroyed: losses of 10–15% for serine and 5–10% for threonine	• Reduce hydrolysis time • Use laboratory-dependent CFs	Ozols (1990); Darragh and Moughan (2005); Rowan et al. (1989); Bech-Andersen (1991)
Tyrosine	Halogenation of tyrosine Decreasing of yield recovery in presence of iron or copper ions Decreasing of yield recovery in presence of fat content	• Add phenol to 6 M HCl • Use constant boiling HCl • Defat sample before acid hydrolysis	Nissen (1992); Finley (1985)
Valine and isoleucine	Cleavage of peptide bonds of these amino acids is particularly difficult in standard acid hydrolysis conditions	• Increase hydrolysis time to 72 hours	Rayner (1985)
Lysine and hydroxylysine	In case of food heat-processing, possible Maillard reaction with reducing sugars; these Maillard products are labile in acidic conditions and can revert back to lysine, leading to overestimation of native lysine content	Apply methods using: • FDNB[c] • FDNB-difference • TNBS • sodium borohydride • furosine • dye binding • ninhydrine-reactive lysine • o-phtaldialdehyde-reactive lyine guanidination[c]	Rao, Carter and Frampton (1963); Carpenter and Bjarnason (1968); Hurrell and Carpenter (1974); Desrosiers et al. (1989); Hendriks et al. (1994); Friedman, Pang and Smith (1984); Vigo et al. (1992); Mauron and Bujard (1964); Mao, Lee and Erbersbobler (1993); Rutherfurd and Moughan (1997); Torbatinejad, Rutherfurd and Moughan (2005)

CF: conversion factor: FDNB: 1-fluoro-2,4-dinitrobenzene; TNBS: 2,4,6-trinitrobenzene sulfonic acid.

[a] Barium hydrolysis forms precipitate over time as barium carbonate or barium sulfate if sulfates are present.

[b] Few laboratories use this method.

[c] These methods are the most commonly used.

Source: adapted from Rutherfurd and Gilani (2009).

Other amino acid analysis techniques involve precolumn derivatization of the free amino acids followed by chromatography (Dai et al., 2014). Precolumn derivatization techniques are sensitive and usually require 0.5–1.0 µg of protein sample per analysis, but may be influenced by buffer salts in the samples. The most commonly used chromatography for amino acid analysis with precolumn derivatization is reverse-phase chromatography (RP-HPLC), but hydrophilic-interaction liquid chromatography provides an alternative approach (Buszewski & Noga, 2012; Alpert, 1990; Hemstrom & Irgum, 2006; de Castro & Sato, 2015; La Barbera et al., 2017; Sánchez-Rivera et al., 2014; Stodt & Engelhardt, 2013). Gas chromatography and liquid chromatography coupled with mass spectrometry are also applied to amino acid analysis (Merrick et al., 2011; Przyborowska et al., 2004).

Precolumn derivatization techniques may result in multiple derivatives of a given amino acid, which makes interpretation of the results more challenging. Silylation is the most commonly used derivatization technique, although it has been reported that alkylation with methyl chloroformate improves analytical performance (Smart et al., 2010; Villas-Bôas et al., 2011). The instability problems of derivatization reagents have been largely eliminated by online derivatization. Numerous strategies for derivatization have been developed and published over recent decades (**Table 8**) (Callejon, Troncoso & Morales, 2010; Fekkes, 1996; Molnár-Perl, 2003). The final performance of the liquid or gas chromatography depends on the efficiency of the derivatization procedure, and subpicomole quantities of protein can be accurately analysed (Fekkes, 1996).

Numerous studies have been published on protein and amino acid analysis by liquid or gas chromatography (coupled or not coupled with mass spectrometry), including studies on milk or dairy products (Marino et al., 2010) and soybean or soy products (**Table 9**). Gas chromatography generally gives lower relative standard deviations (i.e. is more accurate) than liquid chromatography, while only high-resolution UHPLC (UHPLC-Q-Orbitrap, HRMS) provides analysis close to gas chromatography.

Table 8. Main derivatization methods based on specific amine or amide reagents for amino acids analysis

REAGENT OF DERIVATIZATION	SENSITIVITY RANGE	REFERENCES
POST-COLUMN		
Ninhydrin	10 pmol – 50 pmol	Samejima et al. (1971); Moore and Stein (1948; 1954)
OPA Fluorometric	5–10 times more sensitive than ninhydrin	Benson and Hare (1975); Lee and Drescher (1978)
PRECOLUMN		
Phenylisothiocyanate	–	Bidlingmeyer et al. (1987); Bidlingmeyer, Cohen and Tarvin (1984)
AQC	40–800 fmol, depending on the amino acid	Cohen and Michaud (1993)
DABS-Cl	–	Akhlaghi et al. (2015)
FMOC-Cl	Low fmol range (very quick reaction, 30 sec to 1 min)	Einarsson, Josefsson and Lagerkvist (1983)
NBD-F	2.8–20 fmol	Aoyama et al. (2004)

AQC: 6-aminoquinolyl-N-hydroxysccinimidyl carbamate; DABS-Cl: 4-N,N-dimethylaminoazobenzene-4'-sulfonyl chloride; FMOC-Cl: fluorenylmethyl chloroformate; NBD-F: 4-fluoro-7-nitro-2,1,3-benzoxadiazole; OPA: o-phthalaldehyde.

Source: adapted from Rutherfurd and Gilani (2009).

Table 9. A selection of studies specifically related to amino acid analysis in milk and soybean products by gas chromatography and liquid chromatography

PRODUCT OR MATRIX	TECHNIQUE	AMINO ACIDS DETERMINED (RESIDUES %)	REFERENCE
SOYBEAN			
GC-FID and RP-HPLC	GC	Ala (2.2); Arg (ND); Asp (1.0); Cys (8.6); Glu (1.2); Gly (1.3); His (ND); Ile (1.6); Leu (0.7); Lys (3.1); Met (22.4); Phe (4.4); Pro (1.1); Ser (1.6); Thr (1.0) ; Tyr (3.4); Val (1.1)	Adeola, Buchanansmith and Early (1988)
	LC	Ala (3.3); Arg (12.3); Asp (4.9); Cys (6.7); Glu (5.4); Gly (2.4); His (10.4); Ile (3.3); Leu (1.8); Lys (6.5); Met (23.5); Phe (5.6); Pro (2.2); Ser (3.8); Thr (3.4); Tyr (6.9); Val (12.1)	
RP-HPLC		Ala (2.6); Arg (3.2); Asp (4.5); Cys (ND); Glu (3.0); Gly (3.8); His (12.6); Ile (2.5); Leu (3.2); Lys (6.2); Met (15.0); Phe (7.8); Pro (6.0); Ser (5.5); Thr (5.8); Trp (ND); Tyr (2.5); Val (0.8)	Chang, Skauge and Satterlee (1989)
LC-MS-MS		Ala (4.38); Arg (3.98); Asp (3.55); Cys (33.89); Glu (3.77); Gly (10.15); His (9.89); Ile (3.79); Leu (2.65); Lys (6.04); Met (6.68); Phe (2.69);Pro (6.57); Ser (7.07); Thr (5.59); Trp (8.28); Tyr (7.51); Val (2.18); NH3	Zarkadas et al. (2007)
RP-HPLC-Fluo		Ala (4.6); Arg (7.1); Asp+Asn(11.9); Cys (1.2); Glu+Gln (19.0); Gly (4.5); His (3.1); Ile (4.7); Leu (8.0); Lys (6.1); Met (1.8); Phe (5.6); Pro (5.6); Ser (4.8); Thr (4.0); Tyr (2.9); Val (5.3)	Kwanyuen and Burton (2010)
MILK			
RP-HPLC-Fluo		Ala (6.04); Arg (6.12); Asp (4.29); Cys (24.7); Glu (1.73); Gly (3.11);His (1.79); Ile (5.80); Leu (0.72); Lys (3.76); Met (12.08); Phe (1.28); Pro (7.17); Thr (10.03); Tyr (14.08); Val (7.93)	Hejtmankova et al. (2012)
RP-HPLC-DAD		Ala (6.7); Arg (6.2); Asp (5.5); Glu (5.5); Gly (6.4); His (7.6); Ile (6.2); Leu (6.2); Lys (6.6); Met (23.0); Phe (6.6); Pro (6.4); Ser (6.4); Thr (6.2); Tyr (6.5); Val (5.8)	Lu et al. (2015)
UHPLC-Q Orbitrap HRMS		Arg (9.6); Asp (2.7); Asp (5.1); Cys (3.4); Glc (1.3); Glu (0.2); Gly (3.8); His (0.3); Ile (0.2); Leu (2.7); Lys (0.6); Met (3.5); Phe (2.6); Pro (1.1); Ser (2.0); Thr (3.8); Trp (7.4); Tyr (4.9); Val (5.0)	Yin et al. (2016)

GC: gas chromatography; GC-FID: gas chromatography flame ionization detector; LC: liquid chromatography; LC-MS-MS: liquid chromatography–tandem mass spectrometry; ND: not detected; RP-HPLC: reverse-phase high-performance liquid chromatography; RP-HPLC-Fluo: RP-HPLC fluorescence; RP-HPLC-DAD: RP-HPLC diode array detector; UHPLC-Q Orbitrap HRMS: ultra-high-performance liquid chromatography-quadrupole-orbitrap high-resolution mass spectrometry.

Amino acids: Ala: alanine; Arg: arginine; Asp: aspartate; Cys: cysteine; Glu: glutamate; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Tyr: tyrosine; Val: valine.

4.2.2.3 Capillary electrophoresis

Capillary electrophoresis refers to different techniques that operate in liquid media and use capillary columns in which solvated ions, ionized species and neutral species migrate with different velocities (Righetti, 2005). They can be separated under the action of an electric field and pH-controlled conditions. Different modes of electromigration exist with some applications in food analysis, including protein and amino acid analysis (Nazzaro et al., 2012; Blatný & Kvasnicka, 1999; Hiroyuki & Terabe, 1996; Terabe, 2009; Mala, Gebauer & Bocek, 2016; Svec, 2009; Mikšík, 2018; Kenndler, 2014a; de Oliveira et al., 2016; Voeten et al., 2018; Castro & Manz, 2015; Zhu, Lu & Liu, 2012; Mala, Gebauer & Bocek, 2015; Kenndler, 2014b).

Proteins and amino acids have traditionally been separated by polyacrylamide gel electrophoresis (PAGE); more recently, fast capillary electrophoresis separation of amino acids, with minimal or even no sample pretreatment, was developed for plasma and urine analysis (Kitagishi & Shintani, 1998; Martin-Girardeau & Renou-Gonnord, 2000; Soga & Heiger, 2000). The latter is also a convenient method for food quality control, including for analysis of proteins in milk and soybean products (Castro-Puyana et al., 2012; Otter, 2012; Jager, Tonin & Tavares, 2007; Zhu et al., 2012; Lara et al., 2006; Santos et al., 2005; Meng et al., 2009; Bailon-Perez et al., 2007; Garcia-Ruiz et al., 2006; Erny et al., 2008).

Capillary electrophoresis instruments are sensitive, selective, inexpensive and easy to use for a wide variety of applications (Castro-Puyana et al., 2012; El Deeb, Iriban & Gust, 2011; Garcia-Canas et al., 2014; Kaisoon, Siriamornpun & Meeso, 2008; Andree et al., 2012; Nazzaro et al., 2012; Pinero, Bauza & Arce, 2011). Advances in microchip-channel fabrication now allow analysis to be completed in a shorter time (Culbertson, Jacobson & Ramsey, 2000; Nouadje et al., 1995; Thorsén & Bergquist, 2000; Chen, Warren & Adams, 2000; Thorntion, Fritz & Klampfl, 1997). These techniques are promising for amino acid analysis. A comparison between different separation methods used in amino acid analysis is shown in **Table 10**.

Table 10. Comparison of routine methods of food protein analysis

FACTORS CONSIDERED	PAGE	RP-HPLC	Conventional CE	Microfluidic CE
Time for setting gel or regenerating column	60 min	10 min	2–3 min	2–3 min
Sample extraction	Depending on source	Depending on source	Depending on source	Depending on source
Run time	30–240 min	10–90 min	10 min	1–3 min
Visualization of proteins	2–12 hr	Instant	Instant	Instant
Throughput in 24 hr	20/gel	30	100	300
Health risks for operator	Moderate	Low	Low–medium	Low
Cost of equipment	Low	High	High	Medium
Cost of consumables	Low	Medium	Medium	Low–medium

CE: capillary electrophoresis; PAGE: polyacrylamide gel electrophoresis; RP-HPLC: reverse-phase high-performance liquid chromatography.

Source: adapted from Kaisoon et al. (2008).

4.2.2.4 Protein sequencing

Protein sequencing is the determination of the amino acid sequence of a protein, and can include the characterization of post-translational modifications. The two major methods of protein sequencing are the traditional Edman degradation and the more recent mass spectrometry. In the Edman degradation, the phenyl isothiocyanate reacts with the amine group of the N-terminal amino acid, which can be separated and identified by chromatography (Niall, 1973). More recently, analysis by a tandem mass spectrometer has been applied to the sequencing of peptides by using database search and de novo sequencing (Webb-Robertson & Cannon, 2007; Lu, 2004). Calculation of NPCFs from protein sequence data has been applied to milk protein only (Maubois & Lorient, 2016).

4.2.3 Other methods for protein analysis

4.2.3.1 Colorimetric methods for total protein content analysis

Colorimetric methods for total protein content include Biuret, Lowry assay, bicinchoninic acid (BCA) assay and Bradford (CBB G-250 dye binding).

In the Biuret method, in an alkaline medium the peptide bond forms a blue-violet (purple) complex with copper II ions (Cu^{2+}), whose colour is proportional at 540 nm to the protein concentration (Gornall et al., 1949). The Biuret method is relatively fast and inexpensive but has a high quantitation threshold (about 1 mg/L) (Chebaro et al., 2017; Doumas et al., 1981a; Doumas et al., 1981b; Sapan & Lundblad, 2015). Different interferences have been reported (Sapan & Lundblad, 2015), and the Biuret protein assay cannot be used for most dairy products because lactose interferes in the assay (Finete et al., 2013; Keller & Neville, 1986; Sapan & Lundblad, 2015). Several publications have compared the Biuret method with other protein determination methods (Lott, Stephan & Pritchard, 1983; Gadsden, 1983; Eckfeldt, Kershaw & Dahl, 1984; Mohammad & Stomer, 1991; George & O'Neill, 2001; Briend-Marchal, Medaille & Braun, 2005; Martina & Vojtech, 2015; Katsoulos et al., 2017).

In the Lowry method, proteins react first with an alkaline copper reagent and then with a second reagent comprising a mixture of sodium tungstate and sodium molybdate dissolved in phosphoric acid and HCl (Lowry et al., 1951; Chebaro et al., 2017). This reagent allows the reduction of aromatic amino acids (tyrosine and tryptophan), leading to the formation of a dark blue coloured complex whose absorbance is measured between 650 and 750 nm. The Lowry method has a higher sensitivity than the Biuret method (100-fold more sensitive), and can thus be used to carry out measurements in low protein mixtures or in relatively dilute solutions.

The BCA method is a modification of the Lowry assay (Sarwar et al., 1983a). It is a copper-binding method with optimal conditions for maximizing colour absorbance at 562 nm being 60 °C and pH of 11.25 (Owusu-Apenten, 2002). The relationship between protein concentration and absorbance is nearly linear over the range 0.02–2 µg/µL (Bainor et al., 2011; Walker, 2002). However, many compounds interfere with this method, and it is not well-suited for total protein determination in routine analysis (**Table 11**) (Sarwar et al., 1983a; Owusu-Apenten, 2002).

Table 11. Main interfering compounds for BCA protein assay

INTERFERING COMPOUNDS	REFERENCES
Biogenic amines: dopamine, norepinephrine, tyrosine, serotonin (5-HT), tryptophan	Owusu-Apenten (2002); Slocum and Deupree (1991)
Buffers interfering with BCA: Ada, Ampso, Bes, Bicine, Bistris, Caps, Epps, Hepes, Hepps, Mes, Mops, Pipes, Tes, Benedict-positive compounds Buffers not interfering with BCA: tricine	Lleu and Rebel (1991); Kaushal and Barnes (1986)
Acetol, aminophenol, ascorbic acid, 2,3-butanedione, glucose, glyoxal, 2,4-dinitrophenylhydrazine, pyruvic aldehyde	Chen et al. (1990)
Drugs: chlorpromazine, caffeine (—), carbachol (—), chloramphenicol (—), codeine phosphate (—), lidocaine (—), penicillin G, paracetamol	Marshall and Williams (1991)
Lipids: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, cardiolipin, sphingomyelin	Kessler and Fanestil (1986)
Phenols: gallic acid, tannic acid, pyrogallic acid, pyrocatechol	Kamath and Pattabiraman (1988)
Reducing sugars (e.g. glucose, fructose); nonreducing sugars (sucrose)	Sapan and Lundblad (2015)

BCA: bicinchoninic acid.

In the Bradford assay, binding of the dye Coomassie® Brilliant Blue to protein in an acidic solution leads to a concomitant absorbance shift from 465 nm to 595 nm (Antonio Agustí & Pío Beltrán, 1982). This detection method can also be used in sodium dodecyl sulfate PAGE (SDS-PAGE) gel or other matrices where protein concentration can be determined by densitometry (Bonde, Pontoppidan & Pepper, 1992; Sapan & Lundblad, 2015). Coomassie Brilliant Blue binds to proteins approximately stoichiometrically. The relationship is linear and a regression curve can be derived from a series of standards over a range of 0.02–0.50 mg/mL (Okutucu et al., 2007; Moore et al., 2010). The method is affected by different factors, including detergents, pH and polyphenols (tannins) of high molecular weight (Whiffen, Midgley & McGee, 2007; Martens, Reedy & Lewis, 2004; Redmile-Gordon et al., 2013; Robinson, 1979; Antonio Agustí & Pío Beltrán, 1982) (**Table 12**). Amino acids, peptides and low molecular weight proteins (<3000 Da) are not detected by this method.

Table 12. Main compounds interfering with the Bradford reagent (Coomassie Brilliant Blue G250) or with proteins in samples, and incompatible sample preparation methods

INTERFERING COMPOUNDS	REFERENCES
Plant polysaccharides, tannins, glycosaminoglycans (heparin)	Godshall (1983); Khan and Newman (1990)
Chlorophyll, pectins, ethanol, ionic and nonionic surfactants, lipids, flavonoids	Owusu-Apenten (2002)
Protein glycosylation	Khan and Newman (1990); Fountoulakis, Juranville and Manneberg (1992)
Glycation of human serum	Brimer et al. (1995)
INCOMPATIBLE SAMPLE PREPARATION METHODS	
Sodium deoxycholate – trichloroacetic acid (DOC-TCA) precipitation used to isolate sample protein before analysis, because of the formation of precipitate that interferes with this method	Owusu-Apenten (2002); Chiappelli, Vasil and Haggerty (1979); Pande and Murthy (1994)

In one study, the different methods were compared and applied to the same pooled plasma sample; each assay was found to have advantages and disadvantages in terms of sensitivity, performance, accuracy, and reproducibility or coefficient of variation (CV) (Okutucu et al., 2007) (**Table 13** and **Table 14**). All the methods had a CV of less than 6%.

Table 13. Linearity range and limit of detection of most used colorimetric assays for protein determination

ASSAY	REGRESSION COEFFICIENT (standard graphs)	LINEARITY RANGE (mg/mL)	LIMIT OF DETECTION (mg/mL)
Lowry	Y=1.5473x	0.02 – 0.5	0.015
Bradford	Y=1.677x	0.02 – 0.5	0.018
Biuret	Y=0.15x	0.5 – 4.0	0.02

Source: adapted from Okutucu et al. (2007).

Table 14. Protein concentrations determined by most used colorimetric assays for standard protein samples

ASSAY	SAMPLE PROTEIN CONCENTRATION		
	Plasma, n=7 (graph) (mg/mL)	BSA standard[a] (formula) (mg/mL)	HSA standard[b] (formula) (mg/mL)
Lowry			
Median ± SD	110.2 ± 4.09	109.1 ± 4.31	80.3 ± 2.57
CV %	3.7	3.9	3.2
Bradford			
Median ± SD	95.1 ± 3.3	89.5 ± 3.1	72.5 ± 2.5
CV %	3.5	3.5	3.5
Biuret			
Median ± SD	88.8 ± 3.54	80.9 ± 3.67	81 ± 3.98
CV %	4	4.5	4.9

BSA: bovine serum albumin; CV: coefficient of variation; HSA: human serum albumin; SD: standard deviation.

[a] BSA concentration for Biuret assay 0.5 mg/mL; Lowry, Bradford assays were 0.1 mg/mL.

[b] HSA concentration for Biuret assay 0.5 mg/mL; Lowry, Bradford assays were 0.05 mg/mL.

Source: adapted from Okutucu et al. (2007).

4.2.3.2 Spectroscopic methods for total protein content analysis

Spectroscopic methods for total protein content include ultraviolet (UV)-visible light spectrophotometry and near infrared reflectance (NIR) spectroscopy.

UV-visible light spectroscopy exploits the emission and absorption of UV-visible light and provides information on electronic interactions (Simonian, 2002; Noble & Bailey, 2009; Goldring, 2012; 2015). For protein measurement, UV-visible light spectroscopy measures the characteristic absorption of tyrosine and tryptophan at 280 nm (Warburg & Christian, 1942). The method is subject to many limitations because different proteins have different amounts of these amino acids, other molecules (including nucleic acids) also absorb at 280 nm, and different factors also interfere (Ayers et al., 1998). However, under controlled conditions the measurement of UV absorbance of protein extract solutions from milk, beef, flour, bean, and egg yolk were strongly correlated with Kjeldahl analysis (Beavers et al., 1973; Nakai & Le, 1970; Hambraeus et al., 1976).

Both NIR (0.7–2.7 µm or 14 285–4000 cm^{-1}) and mid-infrared reflectance (MIR) (2.5–25 µm or 4000–400 cm^{-1}) spectroscopy have been used for protein analysis (Bailon-Perez et al., 2007; Linker & Etzion, 2009; Moore et al., 2010; Barbano & Lynch, 2006; Diniz et al., 2011; de Marchi et al., 2014; Porep, Kammerer & Carle, 2015; López-Lorente & Mizaikoff, 2016; Grassi & Alamprese, 2018; Pasquini, 2018; Wiercigroch et al., 2017). The protein concentrations in fluid samples, including milk and soybean, are measured using multivariate analysis and calibration factors, by directly measuring transmitted radiation at 1550 cm^{-1}, which is characteristic of the peptide bond (Ribadeau-Dumas & Grappin, 1989) (Barbano & Lynch, 2006).

Despite various limitations, the NIR method has been applied to milk and dairy products (Moore et al., 2010; de Marchi et al., 2014; McDermott et al., 2017) and was found to be comparable with the Kjeldahl method (Luginbühl, 2002; Sorensen, Lund & Juul, 2003; Etzion et al., 2004; Bonfatti, Di Martino & Carnier, 2011). Measurement of NIR diffuse-reflectance is also used for routine protein analysis of grains (Wetzel, 1983). This method has also

been used to determine the content of fat, protein and total solids in cow's milk cheeses (Rodriguez-Otero, Hermida & Cepeda, 1995), and for protein measurement in soy products (Ferreira et al., 2014; Dong et al., 2018). However, NIR is a secondary technology requiring calibration with standards of known protein content; hence, its effectiveness depends on the quality of the reference material.

4.3 NPCFs for milk-based and soy-based foods

The values of the different CFs (K´, K_A and K_B) calculated for milk-based foods and soy-based foods as identified in the literature review are, respectively, listed in **Table 15** and **Table 16**; the respective dispersions of data are shown in **Fig. 3** and **Fig. 4**.

4.3.1 Conversion factors for dairy-based foods

Only two studies or data sets reported conversion factors that were assessed directly in formulas for infants and children (12 samples). A total of 24 studies or data sets reported data for dairy-based foods and ingredients (**Table 15**).

Table 15. NPCFs reported for dairy-based foods and food ingredients

FOOD	K´	K_A				K_B	REFERENCE
		(a)	(b)	(c)	(d)		
Cow's milk	6.38[a]	–	–	–	–	–	Jones (1931)
	6.36[b]	–	–	–	–	–	Van Boekel and Dumas (1987)
	–	6.32	6.01	–	–	–	Derham (1982)
	–	–	–	6.02	–	–	Sosulski and Imafidon (1990)
	–	–	–	5.99	–	–	Sosulski and Imafidon (1990)[c]
	–	–	–	–	–	5.56	Fujihara et al. (2010)
	–	6.13	5.82	6.08	(6.85)	–	Featherston et al. (1964)[c]
	–	6.07	5.79	5.97	(6.70)	–	Krizova et al. (2013)[c]
	–	5.92	5.66	5.83	(6.52)	–	Ceballos et al. (2009)[c]
	–	6.01	5.71	6.00	(6.74)	–	Marino et al. (2010)[c]
	–	5.99	5.75	5.85	(6.55)	–	Zándoki et al. (2006)[c]
	–	6.11	5.79	6.11	(6.89)	–	(Csapo-Kiss et al., 1994)[c]
Pure milk	–	6.15	5.83	6.04	(6.79)	–	Japan Vegetable Food Associationc
Milk protein isolate	–	6.13	5.86	6.10	(6.75)	–	Rutherfurd and Moughan (1998)[c]
Skim milk powder	–	–	–	6.13	(6.91)	5.75	Boisen et al. (1987)
	–	6.09	5.80	6.10	(6.77)	–	Boisen et al. (1987)[c]
Casein	6.38[a]	–	–	–	–	–	Hammersten (1883)
	–	6.37	6.08	–	–	–	Boisen et al. (1987)
	–	–	–	6.15	–	–	Sosulski and Imafidon (1990)
	–	6.16	5.87	6.01	(6.83)	–	Sarwar et al. (1983b)[c]
	–	6.15	5.88	6.03	(6.78)	-	Tomotake et al. (2001)[c]
(Lactic casein)	–	6.06	5.79	6.05	(6.69)	–	Rutherfurd and Moughan (1998)[c]
α s1–casein	6.36[b]	–	–	–	–	–	Van Boekel and Dumas (1987)
B-casein	6.37[b]	–	–	–	–	–	Van Boekel and Dumas (1987)
β-Lactoglobulin	6.29[b]	–	–	–	–	–	Maubois and Lorient (2016)
α–Lactalbumin	6.25[b]	–	–	–	–	–	
Bovine serum albumin	6.07[b]	–	–	–	–	–	

FOOD	K´	K_A				K_B	REFERENCE
		(a)	(b)	(c)	(d)		
Infant formula	6.38[b]	–	–	–	–	–	Maubois and Lorient (2016)
Child formula powder	–	6.05	–	–	–	–	Nestec
Infant elemental powder	–	5.97	–	–	–	–	
Adult nutritional RTF, high-protein	–	5.57	–	–	–	–	
Adult nutritional RTF, high-fat	–	6.17	–	–	–	–	
Infant formula RTF, milk-based	–	6.15	–	–	–	–	
NIST SRM 1849a	–	6.16	–	–	–	–	
Infant formula powder, partially hydrolysed, milk-based	–	6.08	–	–	–	–	
Toddler formula powder, milk-based	–	6.11	–	–	–	–	
Infant formula powder, milk-based	–	6.09	–	–	–	–	
Adult nutritional powder, low-fat	–	5.73	–	–	–	–	
Child formula powder	–	6.06	–	–	–	–	
Infant elemental powder	–	6.00	–	–	–	–	
Infant formula powder, FOS/GOS-based	–	6.11	–	–	–	–	
Infant formula powder, milk-based	–	6.14	–	–	–	–	
Infant formula RTF, milk-based	–	6.17	–	–	–	–	
Adult nutritional RTF, high-protein	–	5.57	–	–	–	–	
Adult nutritional RTF, high-fat	–	6.18	–	–	–	–	
Milk products	–	–	–	–	–	5.33	Salo-väänänen and Koivistoinen (1996)
Cheddar cheese	6.39[b]	–	–	–	–	–	Rouch et al. (2008)

NIST: National Institute of Standards and Technology; NPCF: nitrogen to protein conversion factor; RTF: ready-to use therapeutic food; SRM: Standard Reference Materials.

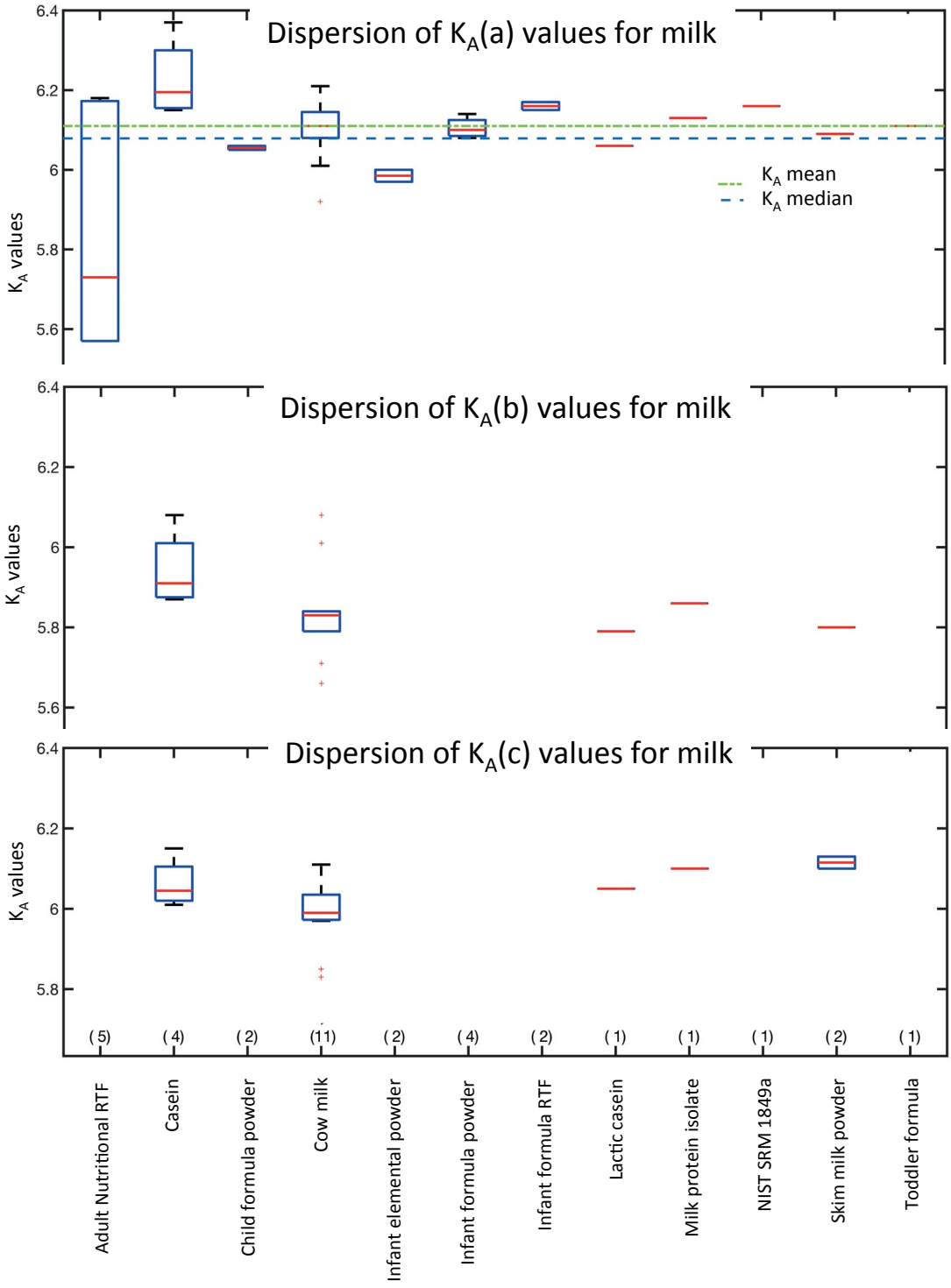
KA(a) assumed amide/acid composition of 50% amide/50% acid; KA(b) assumed amide/acid composition of 75% amide/25% acid; KA(c) amide/acid composition that was directly assessed; KA (d) assumed no difference in nitrogen content between amide and acid forms (not adjusted) – values with parentheses are overestimated and not further used in the calculation.

[a] Weight of the purified protein.

[b] Sum of anhydrous amino acids residues plus weight of the prosthetic group when present.

[c] Specific calculation for this review either by recalculation from previously published calculation, or by calculation using amino acid composition.

Fig. 3 Dispersion of calculated K_A values for dairy protein



NIST: National Institute of Standards and Technology; RTF: ready-to use therapeutic food; SRM: Standard Reference Materials.

K_A(a) assumed amide/acid composition of 50% amide/50% acid; K_A(b) assumed amide/acid composition of 75% amide/25% acid; K_A(c) amide/acid composition that was directly assessed.

Source: values from **Table 15**.

4.3.2 Conversion factors for soy-based foods

Only two data sets reported conversion factors that were assessed directly in formulas for infants and children (four samples). A total of 23 studies or data sets reported data for soy-based foods and food ingredients (**Table 16**).

Table 16. NPCFs reported for soy-based foods and food ingredients

FOOD	K´	K_A					K_B	REFERENCES
		(a)	(b)	(c)	(d)	(e)		
Soybean	5.71[a]	–	–	–	–	–	–	Jones (1931)
	–	–	–	5.63	–	–	5.22(c)	Sosulski and Holt (1980)
	–	5.65	5.38	5.63	(6.28)	–	5.22(c)	Sosulski and Holt (1980)[b]
	–	5.77	5.49	–	–	–	–	Derham (1982)
	–	–	–	–	–	–	5.51(c)	Fujihara et al. (2010)
Soybean meal	–	–	–	5.69	–	–	–	Tkachuk (1969)
	–	–	–	5.71	–	–	–	Sarwar, Sosulski and Bell (1973)
	–	–	–	5.65	(6.3)	–	5.9(c)	Boisen et al. (1987)
	–	5.65	5.38	5.61	(6.26)	–	–	Boisen et al. (1987)[b]
	–	–	–	5.67	–	–	5.38(c)	Mossé (1990)
	–	–	–	5.64	–	–	5.13(c)	Sriperm, Pesti and Tillman (2011)
	–	5.63	5.36	5.52	(6.25)	–	5.13(c)	Sriperm et al. (2011)[b]
	–	5.80	5.51	5.78	(6.48)	–	–	Erasmus et al. (1994)[b]
Soybean proteins	–	5.64	5.36	5.63	(6.29)	–	–	Hughes et al. (2011)[b]
Soybean varieties	–	5.70	5.44	5.76	(6.30)	–	–	Zarkadas et al. (1997)[b]
	–	5.73	5.52	5.71	(6.33)	–	–	Zarkadas et al. (2007)[b]
Soy protein isolate	–	–	–	5.74	–	–	–	Sosulski and Sarwar (1973)
	–	5.69	5.40	5.69	(6.37)	–	–	Tomotake et al. (2001)[b]
	–	–	–	–	–	(6.81)	(6.07)(e)	Morr (1981)
	–	–	–	–	–	(6.84)	(5.64)(e)	
	–	–	–	–	–	(6.74)	(5.94)(e)	
	–	–	–	5.77	–	–	5.76(e)	Morr (1981); Morr (1982)
	–	–	–	5.8	–	–	5.79(e)	
	–	–	–	5.7	–	–	5.7(e)	
Defatted soy flakes	–	5.68	5.39	5.55	(6.34)	–	5.13(e)	Morr (1981); Morr (1982; 1981)Morr (1982)[b]
Acid precipitate soy isolate	–	5.68	5.39	5.68	(6.37)	–	5.04(e)	
Dialysis soy isolate	–	5.68	5.39	5.70	(6.35)	–	4.78(e)	
Commercial isolate	–	5.66	5.38	5.61	(6.32)	–	5.18(e)	
Soy protein isolate	–	5.60	5.33	5.59	(6.24)	–	–	Gorissen et al. (2018)[b]
Soy protein concentrate	–	5.70	5.46	5.69	(6.27)	–	–	Rutherfurd and Moughan (1998)[b]
Soy protein isolate	–	5.68	5.43	5.70	(6.28)	–	–	
Soy cotyledons	–	5.65	5.38	5.64	(6.30)	–	<5.39(a)	ENSA[b]
Soy isolate	–	5.66	5.38	5.65	(6.32)	–	–	Dupont[b]
Soy concentrate	–	5.65	5.38	5.65	(6.38)	–	–	
Soy protein isolate	–	5.67	5.38	5.67	(6.34)	–	–	FAO/WHO (2016b)[b]
Soy protein concentrate	–	5.67	5.38	5.67	(6.34)	–	–	
Soy flakes	–	5.65	5.37	5.66	(6.31)	–	–	

FOOD	K´	K_A					K_B	REFERENCES
		(a)	(b)	(c)	(d)	(e)		
Infant formula, soy-based, powder	–	5.69	5.42	5.43	(6.3)	–	–	Nutrition Research Division, Bureau of Nutritional Sciences, Health Canada[b]
Infant formula, soy-based, liquid	–	5.69	5.42	5.41	(6.32)	–	–	
Pure soy milk	–	5.62	5.36	5.37	(6.23)	–	–	
Pure soy milk	–	5.64	5.36	5.63	(6.28)	–	<5.4(a)	Japan Vegetable Food Association[b]
Infant formula powder, partially hydrolysed, soy-based	–	5.71	–	–	–	–	–	Neste[b]
Infant formula, soy-based, powder	–	5.72	–	–	–	–	–	

ENSA: Endocrine Nurses' Society of Australaia, Inc; FAO/WHO: Food and Agriculture Organization/World Health Organization; NPCF: nitrogen to protein conversion factor

K_A(a) assumed amide/acid composition of 50% amide/50% acid; K_A(b) assumed amide/acid composition of 75% amide/25% acid; K_A(c) amide/acid composition that was directly assessed; K_A (d) assumed no difference in nitrogen content between amide and acid forms (not adjusted) – values with parentheses are overestimated and not further used in the calculation;

K_A(e) hydrated amino acid weights with amide/acid composition directly assessed. For K_B, (c), (e) and (a) are the same as for K_A. The original values of K_A and K_B first determined for several soybean protein products by Morr (1981) were almost 20% overestimated, because free rather than dehydrated amino acid residue molecular weights were used in the calculation. The values were subsequently recalculated and corrected to values in the range 5.7–5.8, as indicated in the table (Morr, 1981; 1982).

[a] Weight of the purified protein. Regarding K´ and inclusion of prosthetic groups for soy, an additional conversion factor with a value of 5.91 was calculated specifically for the soy 7S protein β-conglycinin (Maubois & Lorient, 2016), based on the assumption that all three subunits of β-conglycinin are glycosylated. The authors further use this information to estimate factors for total soy proteins with different 11S/7 S ratios, in the range of 5.69–5.79. These values were not included in the final analysis as they were not directly measured but estimated, based on assumptions made in reports in the literature. In addition, processing of proteins, particularly that of soy protein carried out to deactivate anti-nutrients, can result in removal of prosthetic groups and thus lead to variability in presence of prosthetic groups across samples.

[b] Specific calculation for this review, obtained either by recalculation from previously published calculation or by calculation using amino acid composition.

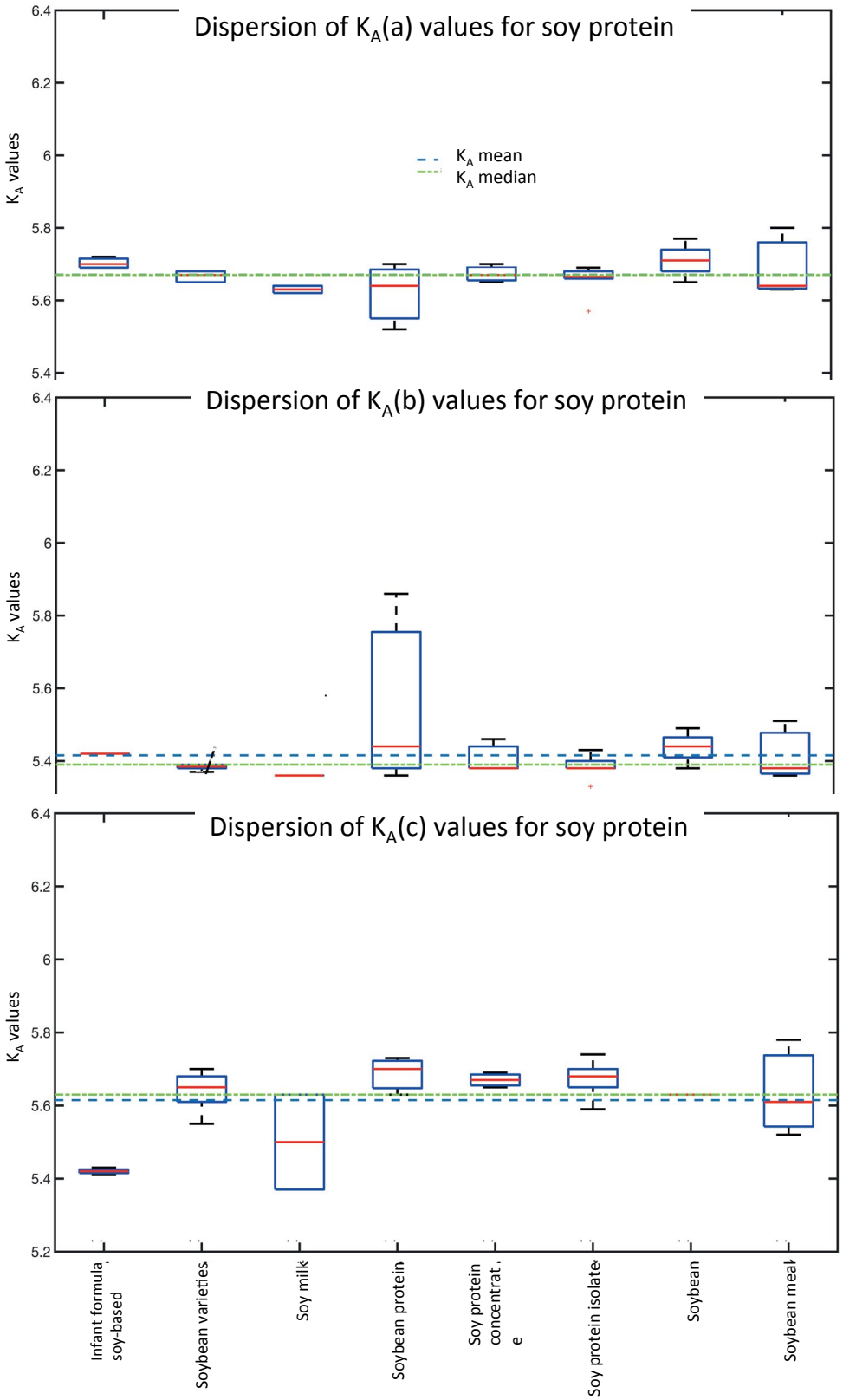
4.3.3 Pooled estimates for NPCFs

The results of pooling the data (separately for dairy and soy) are summarized in **Table 17**. As expected, the observed values for both dairy-based foods and soy-based foods were ordered in the following way: K´ > K_A > K_B; however, only a few studies were identified reporting K´ for soy (N=1), K´ for dairy (N=5 with 10 K´ values reported) and K_B for dairy (N=3). Also, with respect to amide to acid ratios, values for mean K_A(a) = 50/50 and K_A with direct adjustment were similar, with K_A 75/25 being slightly higher than within both dairy and soy assessments.

Only two studies or data sets reported conversion factors that were assessed directly in milk-based formulas for infants and children (12 samples), with a mean value of 6.08 (95% CI: 6.05, 6.12) for K_A 50/50 and a single K´ of 6.32. Mean values across all dairy foods ranged from 5.55 to 6.32, depending on how the CF was calculated.

Only two data sets reported conversion factors that were assessed directly in soy-based formulas for infants and children (four samples), with a mean value of K_A 50/50 of 5.70 (95% CI: 5.69, 5.71), of K_A 75/25 of 5.42, and of K_A direct adjustment of 5.42 (95% CI: 5.40, 5.44). Mean values across all dairy foods ranged from 5.34 to 5.71, depending on how the conversion factor was calculated.

Fig. 4 Dispersion of calculated KA values for soy protein



K_A(a) assumed amide/acid composition of 50% amide/50% acid; K_A(b) assumed amide/acid composition of 75% amide/25% acid; K_A(c) amide/acid composition that was directly assessed.

Source: values from **Table 16**.

Table 17. Pooled estimates for NPCFs

	DAIRY		SOY	
	MEAN (95% CI)	RANGE	MEAN (95% CI)	RANGE
FORMULAS ONLY				
K´	6.38	–	–	–
K_A 50/50	6.08 (6.05, 6.12)	5.97–6.17	5.70 (5.69, 5.71)	5.69–5.72
K_A 75/25	–	–	5.42[a]	–
K_A direct adjust	–	–	5.42 (5.40, 5.44)	5.41–5.43
ALL SAMPLES				
K´	6.32 (6.26, 6.38)	6.07–6.39	5.71[b]	–
K_A 50/50	6.06 (6.00, 6.12)	5.57–6.37	5.68 (5.66, 5.69)	5.60–5.80
K_A 75/25	5.83 (5.77, 5.89)	5.66–6.08	5.40 (5.38, 5.42)	5.33–5.52
K_A direct adjust	6.03 (5.98, 6.07)	5.83–6.15	5.65 (5.61, 5.68)	5.37–5.80
K_B	5.55 (5.31, 5.78)	5.33–5.75	5.35 (5.20, 5.51)	4.78–5.90

CI: confidence interval; NPCF: nitrogen to protein conversion factor.

[a] Two measurements, both with a value of 5.42.

[b] Single measurement only. Regarding K´ and inclusion of prosthetic groups for soy, an additional CF with a value of 5.91 was calculated specifically for the soy 7S protein β-conglycinin (Maubois & Lorient, 2016), based on the assumption that all three subunits of β-conglycinin are glycosylated. The authors further used this information to estimate factors for total soy proteins with different 11S/7S ratios, in the range 5.69–5.79. These values were not included in the final analysis because they were not directly measured but were estimated, based on assumptions made in reports in the literature.

4.3.4 Certainty of the evidence

The certainty in the evidence for NPCFs as assessed by GRADE ranged from very low to moderate. The rating depended on whether protein was defined as amino acids only or amino acids plus prosthetic groups for values of K_A with directly adjusted amide to acid ratios, for both dairy-based foods and soy-based foods; K_B was also assessed as moderate for soy-based foods. Details of the assessments for each of the conversion factors, including rationales for decision-making on each assessment element (i.e. risk of bias, inconsistency, indirectness and imprecision) can be found in the GRADE evidence profiles in **Annex 3**.

4.4 Modelling of non-protein nitrogen

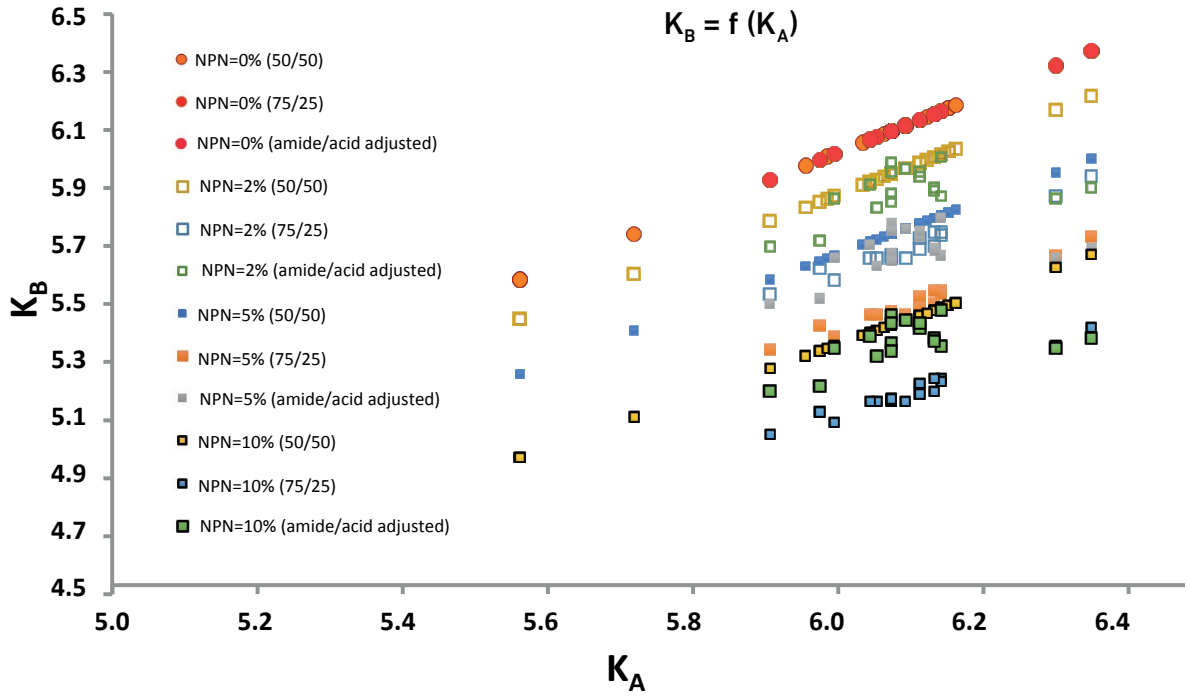
Results of modelling of the impact of non-protein nitrogen on published and calculated K_A values for both dairy-based foods and soy-based foods showed a linear and downward trend, as expected, in modelled K_B values, regardless of the assumptions made for calculating K_A in terms of amide to acid ratio (i.e. 50/50, 75/25 or directly adjusted), and depending on the percentage of non-protein nitrogen entered into the equation (**Fig. 5**). Beginning with 2% non-protein nitrogen, there was a significant difference between K_B and K_A values, increasing with increasing amounts of non-protein nitrogen.

Modelling results for dairy and soy are shown together in **Fig. 6**. The downward trend of K_B was similar for the two product categories, but in the case of milk the impact was slightly more important for a non-protein nitrogen percentage of 10%. The highest variations for K_B were those calculated from a K_A value with 75% amide and 25% acid. The mean value of K_A for milk-based infant formula, as listed in **Table 15**, was 6.09.[1] For this value of K_A, when non-

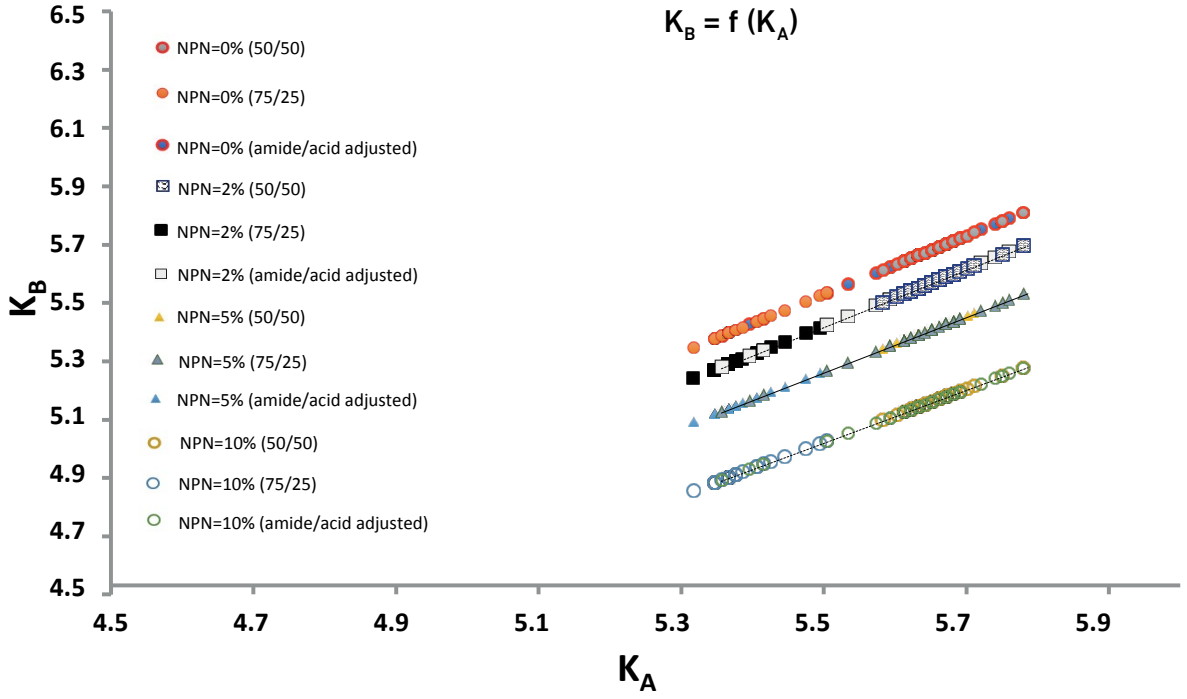
[1] Individual values of 6.05, 5.97, 6.15, 6.08, 6.11, 6.09, 6.00, 6.11, 6.14 and 6.17.

Fig. 5 Relationship between KA and KB according to non-protein nitrogen level for milk-based (A) and soy-based (B) products

A. Milk-based products



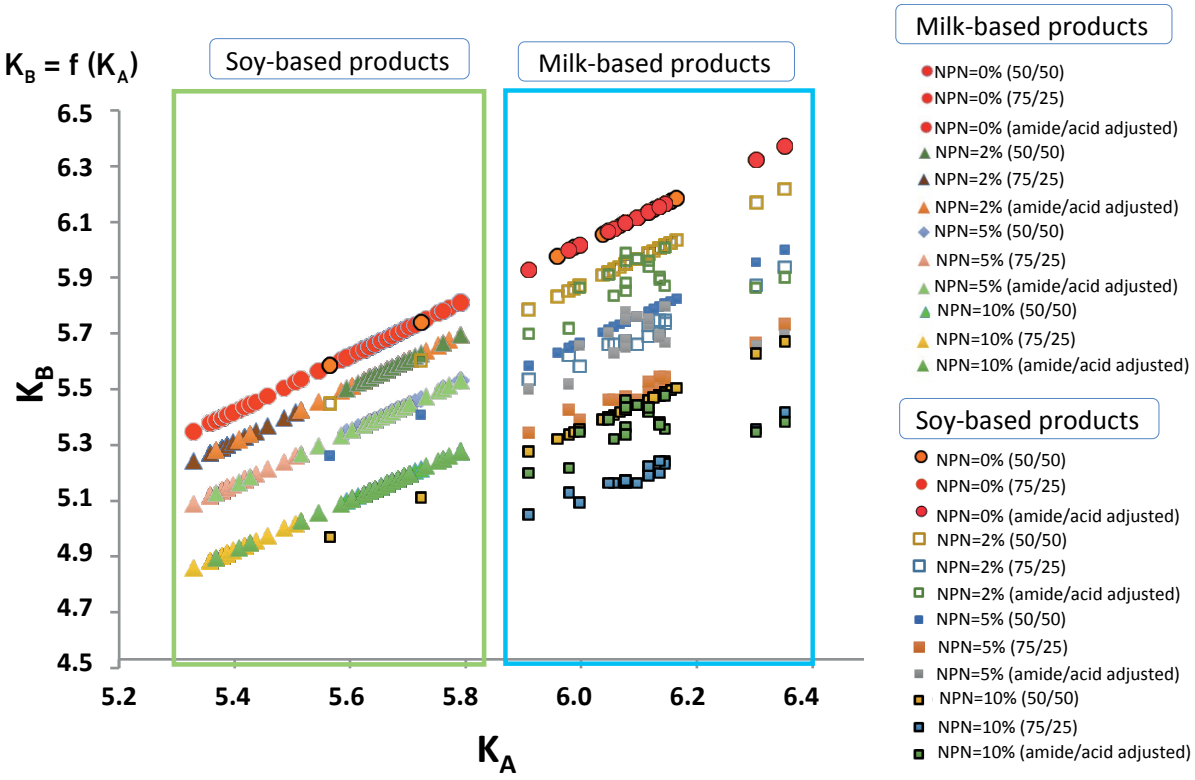
B. Soy-based products



NPN: non-protein nitrogen.

Source: K_A values for milk-based products and soy-based products taken directly from **Table 15** and **Table 16**, respectively.

Fig. 6 All KB values for both soy-based products and milk-based products as a function of non-protein nitrogen



NPN: non-protein nitrogen.

Source: K_A values for milk-based products and soy-based products taken directly from **Table 15** and **Table 16**, respectively.

protein nitrogen varied from 0 to 10% total nitrogen, KB varied from 6.09 to 5.40 (**Table 18**). The mean value of K_A for soy-based infant formula, as listed in **Table 16**, was 5.70.[1] For this value of K_A, when non-protein nitrogen varied from 0 to 10% total nitrogen, K_B varied from 5.70 to 5.1 (**Table 18**).

Table 18. KB values according to non-protein nitrogen content as a function of mean KA valuesa

	MILK-BASED INFANT FORMULA	SOY-BASED INFANT FORMULA
Mean $K_A = K_B$ for 0% NPN	6.09	5.7
K_B for 5% NPN total N	5.7	5.4
K_B for 10% NPN total N	5.4	5.1
Range for K_B in **Tables 15 and 16**	5.3–5.7	4.8–5.9

N: nitrogen; NPN: non-protein nitrogen.

[a] Based on protein weight calculated from the sum of the weights of anhydrous amino acid residues.

[1] Individual values of 5.69, 5.69, 5.71 and 5.72.

5. DISCUSSION

Protein content in food products and food ingredients can be determined either by direct protein analysis or by measuring nitrogen content and using an NPCF (K′, K_A and K_B). The direct measurement of protein can be performed by purification of protein, colorimetric methods, spectrophotometric methods or infrared spectroscopy. These different methods have some limitations, and measuring nitrogen content remains the more frequently used approach for protein content in food products and food ingredients.

5.1 General considerations in calculating and NCPFs

Values for the NPCF K′ were initially proposed based on measurement of total nitrogen content and weight of the different purified proteins (Jones, 1931). Even early on, the results indicated that using a generic conversion factor of 6.25 could lead to a 15–20% error in estimating the actual protein content, and that specific CFs should be applied for the determination of protein content when calculated from the nitrogen content of different food products and food ingredients. Methods for deriving CFs were subsequently improved; for example, basing protein weight on amino acid composition provided more precise values for protein molecular weight and protein nitrogen content (Heathcote, 1950; Tkachuk, 1966b; 1966a; Tkachuk & Mellish, 1977; Sosulski & Holt, 1980; Morr, 1981; 1982; Mossé, 1990).

Although methods have improved, the results of this review and others highlight two critical and limiting considerations in determining appropriate conversion factors for dairy, soy and other proteins, particularly where the purpose of the conversion factor is to provide information primarily on the provision of amino acids and will be used on a large variety of different foods. The first consideration applies to protein with identified prosthetic groups (primarily milk protein), and is whether to include the weight of the prosthetic groups in the calculation of the molecular weight of the protein (Maubois & Lorient, 2016; IDF, 2016). The second consideration applies to food products and ingredients with a significant amount of non-protein nitrogen, and is whether to use K_A (which requires knowing how much protein nitrogen is contained in the sample via non-protein nitrogen measurement and subtracting this fraction to total nitrogen, or by amino acid analysis) or K_B based on total nitrogen content. In addition, the amide to acid ratio must be taken into consideration when calculating and using K_A.

5.1.1 Prosthetic groups

This review found significant differences between K′ and K_A values, resulting from the presence of prosthetic groups on most dairy proteins.

The mean K′ for dairy-based foods is 6.32 (95% CI: 5.67, 6.98) with a range of 6.28–6.39, whereas K_A and K_B, which are based on weights of amino acid residues only, range between 5.33 and 6.15, with means of 5.55–6.11. The K′ values identified in this review were obtained either based on the total nitrogen content and weight of purified acid-precipitated bovine casein or milk protein (Hammersten, 1883; Jones, 1931), or from more recent calculations from amino acid composition and sequence (by adding the weight of the identified prosthetic groups to the weight of the sum of anhydrous amino acids residues) (Van Boekel & Dumas, 1987; Rouch, Roupas & Roginski, 2007; Rouch et al., 2008; Maubois & Lorient, 2016; Karman & van Boekel, 1986).

The presence of prosthetic groups on dairy proteins has been noted in the literature, and can contribute significantly to the measured mass. For example, compared with the weight of proteins assessed as the sum of anhydrous amino acids only, complete phosphorylation of α-casein – which accounts for about half of all dairy protein – can increase the weight by nearly 5% (O'Donnell et al., 2004), and the presence of glycans in lactoferrin can increase its weight by more than 11% (O'Riordan et al., 2014). Total milk protein and casein (which accounts for about 80% of milk protein) have K′ values in the range of 6.34–6.39 owing to prosthetic groups, whereas whey proteins have a lower K′ value (6.29–6.07), probably due to a lower content of prosthetic groups (Maubois & Lorient, 2016).

Although many of the sites for post-translational addition of prosthetic groups in dairy proteins have been characterized, modification at any particular site can vary from sample to sample, making it difficult to precisely assess the contribution of prosthetic groups to the weight of protein in a particular food sample or ingredient. Moreover, for other protein sources, including soy-based food and ingredients, post-translational modifications have not been well characterized, and only K_A or K_B can be calculated from the amino acid composition of the proteins. For soy, the only K′ value identified in the literature review (5.71) was initially calculated based on the measurement of total nitrogen content and weight of purified soybean protein (Jones, 1931).[1] Therefore, until more data are generated for other foods and ingredients, K′ is really only relevant in the context of assessing dairy proteins.

5.1.2 Non-protein nitrogen

K_A values based on protein nitrogen content only are the most widely assessed and published conversion factors (in this review, more than 30 K_A values were identified for both dairy-based and soy-based foods). However, for food products and food ingredients, all but the most highly purified protein samples contain some level of non-protein nitrogen. Therefore, the ability to correct the CF for the presence of non-protein nitrogen in food products and food ingredients is critical when calculating and using conversion factors. Although K_B is the only conversion factor that explicitly takes non-protein nitrogen into consideration, and therefore may provide a truer estimate of protein content, it has proved difficult to precisely define the appropriate value of K_B for most food sources (Heathcote, 1950; Holt & Sosulski, 1979; Morr, 1981; 1982; Sosulski & Imafidon, 1990; Tkachuk, 1969; 1966a; Mossé, 1990). However, to the extent that non-protein nitrogen varies from batch to batch or protein type to protein type for a given class of protein, the factor K_B will introduce inaccuracies.

In this review, only three studies were identified that reported KB values for cow's milk, skim milk powder and various dairy products (Boisen et al., 1987; Fujihara et al., 2010; Salo-väänänen & Koivistoinen, 1996). In milk powders, non-protein nitrogen was measured at a level of about 0.3% weight for weight (DeVries et al., 2017). A greater number of studies were identified that reported on K_B values for soy-based foods. Even without explicit knowledge about the amount of non-protein nitrogen in a food or ingredient, correcting for the content of non-protein nitrogen in these samples by using the factor K_B (and the total nitrogen content of the sample) provides a reasonable estimate of the protein content, particularly in those food

[1] Regarding K′ and inclusion of prosthetic groups for soy, an additional conversion factor with a value of 5.91 was calculated specifically for the soy 7S protein β-conglycinin (Maubois & Lorient, 2016), based on the assumption that all three subunits of β-conglycinin are glycosylated. The authors further used this information to estimate factors for total soy proteins with different 11S/7S ratios, in the range 5.69–5.79. These values were not included in the final analysis because they were not directly measured but were estimated, based on assumptions made in reports in the literature.

products or food ingredients with a significant level of non-protein nitrogen (Templeton & Laurens, 2015; Ezeagu et al., 2002; Fujihara et al., 2010; Jonas-Levi & Martinez, 2017; Mattila et al., 2002; Yeoh & Truong, 1996a; Yeoh & Wee, 1994).

The modelling results presented in this review further support the notion that non-protein nitrogen can have a significant impact on the calculation and subsequent application of conversion factors, particularly when there are significant levels of non-protein nitrogen. Modelling results suggest that for a sample with 10% of total nitrogen content as non-protein nitrogen, the conversion factor can be more than 10% higher when the non-protein nitrogen is not taken into consideration.

5.1.3 Amide to acid ratio

When calculating and using K_A values, nitrogen content must be adjusted for the amide to acid ratio between glutamine and glutamic acid, and between asparagine and aspartic acid. Ways of addressing this include arbitrarily adjusting for amide to acid ratios of 50/50 or 75/25, adjusting based on direct assessment of amide to acid ratio, or ignoring the impact of the amide to acid ratio and not adjusting at all. This review found that for both dairy-based and soy-based food products and food ingredients, values of K_A in which no amide to acid adjustment were made were significantly larger than those where adjustments were made. Because these are generally gross overestimates, they are the least informative of the K_A values. The value obtained using a fixed amide to acid ratio of 50/50 were higher than when using a ratio of 75/25.

The K_A values calculated for cow's milk, skim milk powder and casein were in the range 5.99–6.32 when using a fixed amide to acid ratio of 50/50, 5.66–6.08 when using a fixed amide to acid ratio of 75/25, and 5.99–6.15 after direct amide to acid adjustment. However, the only K_A value of 6.32 obtained using a fixed amide to acid ratio of 50/50 (Derham, 1982) was clearly above the other values obtained using the same ratio; if this value is not taken into account, the K_A values calculated for cow's milk, skim milk powder and casein using a fixed amide to acid ratio of 50/50 were in the range 5.99–6.13 (i.e. very close to the values using the directly measured ratio), whereas lower results were obtained by considering an amide to acid ratio of 75/25. Similarly, results obtained for soy-based foods showed that those values with an amide to acid ratio of 50/50 were close to the values using the directly measured ratio, whereas lower values were obtained by considering an amide to acid ratio of 75/25. Values were also overestimated when the amide to acid ratio was not adjusted, whereas a K_A value of 5.65 was calculated for soybean meal after direct adjustment of the amide to acid ratio (Boisen et al., 1987). Overall, this suggests that when the amide to acid ratio is not directly measured or otherwise known, the estimated ratio of 50/50 might provide a reasonably accurate estimate for both dairy-based and soy-based foods and food ingredients.

5.2 Selecting NPCFs

Selecting one or more conversion factors for dairy and soy-based ingredients to be used as benchmarks across a large number of different samples (in this case infant formula and follow-up formula, comprising different formulations from different manufacturers) requires consideration of both the type of CF to use (i.e. K´, K_A or K_B) and the specific value of the CF. The choice of whether to use K´, K_A or K_B in the case of dairy, or K_A or K_B in the case of soy, depends on both the purpose of using the conversion factor (e.g. is it primarily to assess delivery of amino acids?) and consideration of any potential variation in non-protein nitrogen

content across many different formulations and compositions of infant formula and follow-up formula.

Regarding the purpose of the conversion factor, if the interest is primarily in quantifying the amount of amino acids in a sample, then K_A or K_B are more relevant because they provide information on amino acid content only. If the interest is in determining the total protein content including prosthetic groups, then K´ would be more informative; however, it is essentially only available for dairy proteins. Regarding variation in non-protein content, use of a particular K_B assumes that non-protein nitrogen content of the sample used to derive the particular K_B selected is the same or very similar in all samples that will be assessed using the particular K_B. Otherwise, it would be more appropriate to determine the non-protein nitrogen by subtracting it from the total nitrogen content and applying K_A or K´ to the difference.

5.3 Comparability with other reviews

Other reviews using mainly a narrative approach have examined the values of NPCFs for the calculation of protein content in foods (Mariotti et al., 2008; Maubois & Lorient, 2016; Krul, 2019). The methodological approaches and exclusion criteria used in those reviews differed substantially from our criteria in the present study. However, the conclusions of these different reviews are in good agreement with the present conclusion related to the values calculated for the different conversion factor K´, K_A and K_B, and with the significance of the differences between these different CFs. All the reviews considered that the more accurate approach is to first derive the weight and nitrogen content of the different proteins from their amino acid composition. In the review from Maubois and Lorient (2016), adding the weight of the prosthetic group to calculate the weight of milk protein was preferred, but the values of K´ agreed with other results from references cited here. There was also a general conclusion that the main uncertainty arises from non-protein nitrogen and calculation of K_B.

5.4 Comparability with other conversion factors

The results on conversion factors obtained for milk-based and soy-based foods and food ingredients agree with those obtained for other food products. For comparison, different results on other food products also showed that the K_A values are for many products in the range 5–6, and that $K_A > K_B$ as expected. For instance, for meat, the value of 6.25 for K´ was clearly overestimated: a K_A conversion factor of about 5.7 is more appropriate and a K_B of 5.17 was proposed (Jones, 1931; Holt & Sosulski, 1979; Salo-väänänen & Koivistoinen, 1996; Rafecas et al., 1994). For wheat, K´ was 5.83 and the measured K_A and K_B values were in the ranges 5.14–5.93 and 5.18–5.55, respectively (Jones, 1931; Sosulski & Sarwar, 1973; Mossé, Huet & Baudet, 1985; Fujihara et al., 2008; Liu et al., 2018). For rice, K´ was 5.93 and the measured K_A and K_B values were in the ranges 5.61–5.78 and 4.9–5.35, respectively (Jones, 1931; Sosulski & Sarwar, 1973; Mossé, Huet & Baudet, 1988; Fujihara et al., 2008). For seaweed, K_B was in the range 3.53–5.72 (Angell et al., 2016; Biancarosa et al., 2017; Diniz et al., 2011; Lourenço et al., 2002). For *Palmaria palmata*, K_B was 4.7 (Bjarnadottir et al., 2018). For cassava root, K_A was in the range 4.75–5.87 and K_B was 3.24 (Yeoh & Truong, 1996b). For mushrooms, K_B was in the range 4.5–4.97 (Mattila et al., 2002). For crop residues, K_A was 5.42–6.00 and K_B 3.97–4.57; for animal manure, K_A and K_B were in the ranges 4.78–5.36 and 3.97–4.57, respectively (Chen et al., 2017).

6. CONCLUSION

From the limited number of studies and data sets identified that specifically calculated conversion factors for infant formula or follow-up formula, the following mean values were observed: for milk-based formulas, a mean value of 6.08 (95% CI: 6.05, 6.12; moderate certainty of evidence) for K_A 50/50 and a single K´ value of 6.38 (very low certainty of evidence[1]); and for soy-based formulas, mean values of 5.70 (95% CI: 5.69, 5.71; very low certainty of evidence) for K_A 50/50, 5.42 (very low certainty of evidence) for K_A 75/25, and 5.42 (95% CI: 5.40, 5.44; very low certainty of evidence) for K_A direct adjustment. This review also showed that when broadly considering dairy proteins inclusive of prosthetic groups, mean K´ was 6.32 (95% CI: 6.26, 6.38; low certainty of evidence1). The results further suggested that when considering amino acids alone, without prosthetic groups, and when amino acid composition of the protein source is available (as it is for milk and soy protein), K_A can be accurately calculated with a value in the ranges 5.83–6.06 for milk protein (low to moderate certainty of evidence) and 5.40–5.68 for soy protein (low certainty of evidence). Because these values do not take into consideration non-protein nitrogen, however, they should be considered as the maximum values for milk-based and soy-based foods. In other words, when non-protein nitrogen is considered, K_B will likely be less than or equal to 5.83–6.06 for dairy-based foods and food ingredients, and less than or equal to 5.40–5.68 for soy-based foods and food ingredients. Indeed, we observed a mean K_B of 5.35 (95% CI: 5.20, 5.51) for soy-based foods and food ingredients, with low certainty of evidence,[1] from a fairly large number of studies. K_A can, however, be applied to total nitrogen values corrected for non-protein nitrogen. There were too few values reported for dairy foods to have much confidence in the observed mean K_B of 5.55 (95% CI: 5.31, 5.78; very low certainty of evidence) for dairy. In addition, as presented in this review, alternative methods for protein content should be discussed, including direct protein analysis or direct amino acid analysis. These methods have been improved over time, and direct amino acid analysis could represent an interesting alternative in the future.

[1] Depending on assumptions made regarding definition of protein.

ANNEXES

ANNEX 1.

SEARCH STRATEGY

Table A.1 Keyword combinations used in the database searches of publications (1946–2019)

KEYWORDS COMBINATION	NUMBER OF PUBLICATIONS			
	(1)	(2)	(3)	(4)
("amino acid* composition*" OR "nitrogen to protein conversion factor*" OR "nitrogen conversion factor*") AND ("food*" OR "milk" OR "dairy" OR "soy*" OR "soybean*")	1892	1340	>4000a	>4000a
("amino acid* composition*" OR "nitrogen to protein conversion factor*" OR "nitrogen conversion factor*") AND ("milk" OR "dairy" OR "soy*" OR "soybean*")	1009	577	1874	1244
("amino acid* analysis" OR "nitrogen to protein conversion factor*" OR "nitrogen conversion factor*") AND ("food*" OR "milk" OR "dairy" OR "soy*" OR "soybean*")	480	533	>900a	>900[a]
("amino acid* analysis" OR "nitrogen to protein conversion factor*" OR "nitrogen conversion factor*") AND ("milk" OR "dairy" OR "soy*" OR "soybean*")	247	219	421	286
("nitrogen to protein conversion factor*" OR "nitrogen conversion factor*") AND ("food*" OR "milk" OR "dairy" OR "soy*" OR "soybean*" OR "Protein*")	54	252	44	36
("nitrogen to protein conversion factor*" OR "nitrogen conversion factor*") AND ("milk" OR "dairy" OR "soy*" OR "soybean*" OR "Protein*")	54	212	44	35
("nitrogen analysis") AND ("food*" OR "milk" OR "dairy" OR "soy*" OR "soybean*")	45	26	67	27
("nitrogen analysis") AND ("milk" OR "dairy" OR "soy*" OR "soybean*")	29	16	38	12
Total number of papers from each databaseb	1289	900	2298	1465
Final corpusb	3881			

(1) "Web of Science Core Collection – Clarivate Analytics" and using the field "All fields" for (2) "Medline/PubMed of the National Library of Medicine", (3) "CAB Direct/CAB Abstracts – CABI", and (4) "Food Science and Technology Abstracts – Ebsco/IFIS".

[a] Not included in the final corpus and discarded due to background noise.

[b] Total after removing duplicates within and between databases.

The keywords "food" and "protein", and to a lesser extent "amino acid composition" "amino acid analysis", produced significant background noise.

From the corpus of the **3881 publications**, a series of requests relating to amino acid, nitrogen and conversion factor was constructed (**Table A.2**).

Table A.2 Requests from the corpus of the 3881 publications (1946–2019)

REQUESTS ON THE WORDS OF THE TITLE, THE SUMMARY OR THE KEYWORDS OF THE PUBLICATION	MILK OR DAIRY	SOY	INFANT FORMULA	TOTAL[A]
Amino acid (analysis OR content OR composition)	694	610	13	1274
Nitrogen (analysis OR content OR determination)	34	20	0	53
Conversion factor	4	5	1	8
Total[a]	714	619	14	1316
REQUESTS ON THE WORDS OF THE TITLE OF THE PUBLICATION	**MILK OR DAIRY**	**SOY**	**INFANT FORMULA**	**TOTAL[A]**
Amino acid (analysis OR content OR composition)	177	109	3	285
Nitrogen (analysis OR content OR determination)	3	2	0	5
Conversion factor	3	5	0	6
Total[a]	183[a]	116[a]	3[a]	297[a]
SELECTED PUBLICATIONS FOR NPCFS	–	–	–	67
Unpublished data from the call[b]	2	6	1	5[a]

[a] Total after removing duplicates within and between databases.

[b] After a call for data on amino acid analysis, unpublished data were obtained from Japan Vegetable Food Association (milk and soy), Nestec (milk and soy), Dupont (soy), Nutrition Research Division – Bureau of Nutritional Sciences – Health Canada (soy), ENSA (soy).

An additional series of requests made on the methods for nitrogen and protein analysis, and another on publications compiled in the ISI Web of Knowledge on the words in the title with "analytical methods", "Kjeldahl" and "Dumas method" (1956–2019) or with other sources (e.g. Google Scholar, Science Direct, Wiley) provided 392 publications (Table A.3).

Table A.3 Additional requests from the corpus of the 3881 publications (1946–2019)

REQUEST ON THE WORDS OF THE TITLE, THE SUMMARY OR THE KEYWORDS OF THE PUBLICATION	KJELDHAL	DUMAS	TOTAL[A]
Total initial corpus Milk OR Dairy Soy	65 13 5	3 0 0	65 13 5
Total[a]	65	3	65)
(i) Selected publications from the corpus (ii) Additional publications	33 27	3 17	33 1
(i)+(ii) Total[a]	78[a]	18[a]	85[a]
Domains *Web of Knowledge*	**Kjeldhal**	**Dumas**	**Total[a]**
Food sciences and technology Agronomy Agricultural engineering Plant sciences	374 206 162 146	45 19 4 16	392
Selected publications for analytical methods	**–**	**–**	**215**

[a] Total after removing duplicates within and between databases.

ANNEX 2

EXCLUDED STUDIES

Exclusion of studies was based on analysis of the words from either title, keywords, abstracts or from full text screening.

The reasons for exclusion of studies included the following:

- the studies or the articles are completely outside the scope of the review;
- the studies or the articles refer to NPCFs, for instance in the experimental part, but do not contribute or provide data that can be used in the review;
- the studies or the articles refer to protein analysis but do not contribute or provide data that can be used in the review;
- the studies or the articles refer to amino acid analysis but do not contribute or provide data that can be used in the review;
- the studies or the articles use an NPCF (most often of 6.25) but do not address the calculation and validation of this factor;
- the studies or the articles provide data previously published; and
- some articles were already cited and do not provide additional information.

Regarding studies reporting empirically determined values for NPCFs, specific studies were excluded from this review for the reasons set out below.

Articles addressed products other than milk and soy or were not relevant to the objective of the review. Example articles:

Milton et al. (1981) Biotropica. 13:177–81

Baudet et al. (1986) J Agric Food Chem. 34:365–70

Mossé et al. (1987) J Phytochemistry. 26:2453–8

Fujihara et al. (1995) J Food Sci. 60:1045–7

Lourenço et al. (1998) J Phycol. 34:798–811

Fujihara et al. (2001) J Food Sci. 66:412–5

Lourenço et al. (2004) Eur J Phycol. 39:17–32

Martre et al. (2006) Eur J Agron. 25(2):138–54

Zándoki (2006) Czech J Anim Sci. 51(9):375–82

Zarkadas et al. (2007) Food Research International. 40:129–46

Estrella (2008) Quito Usfq. 5:1–26

Fujihara (2010) J Integrated Study of Dietary Habits. 21:98–106

Colwell et al. (2011) J Assoc Publ Analysts. 39:4–78

Graciela et al. (2011) Am J Plant Sci. 2(3):287–96

Lewis (2012) Br J Nutr. 108(Suppl S2):S212–S21

Hall (2013) Food Chem. 140:608–12

Safi et al. (2013) J Appl Phycol. 25:523–9

Shuuluka et al. (2013) J Appl Phycol. 25:677–85

Diniz et al. (2013) Latin American J Aquatic Res. 41:254–64

Diniz et al. (2014) Latin American J Aquatic Res. 42:332–52

Magomya et al. (2014) Int J Sci Tech Res. 3:68–72

Angell et al. (2014) J Phycol. 50:216–26

Rayaprolu et al. (2015) J AOCS. 92:1023–33

Angell et al. (2015) [data set]. doi:10.4225/28/55776D6F45871

Janssen et al. (2017) J Agric Food Chem. 65:2275–8.

Articles reported an NPCF of 6.25 or another fixed value but did not address the calculation. Example articles:

Roberts & Briggs (1965) Cereal Chem. 42:71

Koshiyama (1968) Cereal Chem. 45:405

Murphy & Resurreccion (1984) J Agric Food Chem. 32:911–5

Gayler & Sykes (1985) Plant Physiol. 582–5

Goedhart & Bindels (1994) Nutr Res Rev. 7:1–23

Solymos & Horn (1994) Acta Vet Hung. 42:487–94

Emmett & Rogers (1997) Early Human Development 49(Suppl):S7–S28

Rand al. (2003) Am J Clin Nutr. 77:109–127

Koletzko & Shamir (2006) Brit Med J. 332:621–2

Jing et al. (2010) Early Hum Devel. 86:119–25

Hall & Schönfeldt (2013) Food Chem. 140:608–12

Andres et al. (2013) J Pediatr. 163:49–54

Pivik et al. (2013) Intl J Psychophysiol. 90:311–20

Vandenplas et al. (2014) Br J Nutr. 111:1340–60

Ziegler et al. (2015) J Pediatr Gastroenterol Nutr. 61:596–603

Elgar et al. (2016) J AOAC Intl. 99:26–9

Articles were already cited by Maubois and Lorient (2016) for the values of non-protein nitrogen. Example articles:

Wolfschoon-Pombo & Klostermeyer (1981) Milchwissenschaft. 36:598–600.

Robertson & Van der Westhuizen (1990) S Afr J Dairy Sci. 22:1–8

ANNEX 3

GRADE EVIDENCE PROFILES

GRADE evidence profile 1 – Dairy-based ingredients

Question: When using the equation *amount of protein (P) = nitrogen to protein conversion factor (K) * amount of nitrogen (N)* to estimate the protein content of dairy-based ingredients used in infant formula and follow-up formula, which value of K most closely estimates the true amount of protein (P), where "protein" is defined as amino acid content only?

Population: Infant formula and follow-up formula

Quality assessment							No. of independent measurements	Mean (95% CI)	Certainty	Importance[1]
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations				
Nitrogen to protein conversion factor K´ (unitless) – all dairy foods										
5	Mixed[2]	Not serious[3]	Not serious[4]	Very serious[5]	Not serious[6]	None	10	6.32 (6.26, 6.38)	⨁⨁◯◯ LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 50/50 (unitless) – all dairy foods										
13	Amino acid analysis[7]	Not serious[3]	Not serious[4]	Not serious[8]	Not serious[6]	None	31	6.06 (6.00, 6.12)	⨁⨁⨁◯ MODERATE[9]	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 75/25 (unitless) – all dairy foods										
12	Amino acid analysis[7]	Not serious[3]	Not serious[4]	Serious[10]	Not serious[6]	None	14	5.83 (5.77, 5.89)	⨁⨁◯◯ LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with directly calculated amide/acid ratio (unitless) – all dairy foods										
12	Amino acid analysis[7]	Not serious[3]	Not serious[4]	Serious[11]	Not serious[6]	None	16	6.03 (5.98, 6.07)	⨁⨁◯◯ LOW	CRITICAL
Nitrogen to protein conversion factor K_B (unitless) – all dairy foods										
3	Amino acid analysis[7]	Not serious[3]	Not serious[4]	Serious[12]	Very serious[13]	None	3	5.55 (5.31, 5.78)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K´ (unitless) – formulas only										
1	Amino acid sequencing[14]	Not serious[3]	Not serious[4]	Serious[15]	Very serious[13]	None	1	6.38 (single measurement)	⨁◯◯◯ VERY LOW	CRITICAL

Quality assessment							No. of independent measurements	Mean (95% CI)	Certainty	Importance[1]
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations				
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 50/50 (unitless) – formulas only										
1	Amino acid analysis[7]	Not serious[3]	Not serious[4]	Not serious[16]	Not serious[6]	None	11	6.08 (6.05, 6.12)	⨁⨁⨁◯ MODERATE	CRITICAL

CI: confidence interval.

[1] Importance for decision-making. In this case, conversion factors are the only "outcome" and thus each of the variations is critical for decision-making.

[2] Amino acid sequencing and measurement (weighing) of crude protein with total nitrogen analysis. Because this factor was derived primarily from studies using amino acid sequencing, it was started at "high" certainty.

[3] There was no indication of systematic measurement error or reporting error (i.e. selective reporting of K´ values). Not downgraded.

[4] Inconsistency was assessed by considering the level of variance around the mean. The 95% CI suggests very little variation around the mean. Not downgraded.

[5] Only a single K´ value for infant formula and follow-up formula was identified in the literature review. The mean conversion factor was therefore primarily derived from dairy sources other than infant formula and follow-up formula (e.g. milk, milk proteins, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. In addition, calculation of K´ includes prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids only. Downgraded twice for very serious indirectness.

[6] The minimum of 10 measurements is satisfied. Not downgraded.

[7] Because these factors were derived primarily from studies using amino acid composition analysis, they were started at "moderate" certainty.

[8] Of the 31 measurements, 11 come from infant formula and follow-up formula. Although K_A 50/50 and 75/25 values are based on setting the ratio of amide to acid arbitrarily at 50/50 or 75/25, it was expected that this would not result in significant enough difference from values derived via direct adjustment. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration in estimating protein content in a variety of formulas with different formulations. There was no indication of reporting error (i.e. selective reporting of K_A values). Not downgraded.

[9] In addition to having greater confidence in the K_A 50/50 value relative to K_A 75/25, because the former includes a significant number of studies that derived conversion factors directly from infant formula and follow-up formula (and therefore was not downgraded for serious indirectness), there was greater confidence in this value because it is in line with that of the K_A derived from the directly adjusted amide to acid ratio, which is the most accurate method of assessing amide to acid ratio.

[10] No studies reported values for infant formula and follow-up formula. The mean conversion factor was therefore derived from dairy sources other than infant formula and follow-up formula (e.g. milk, milk proteins, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Although K_A 50/50 and 75/25 values are based on setting the ratio of amide to acid arbitrarily at 50/50 or 75/25, it was expected that this would not result in significant enough difference from values derived via direct adjustment. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration in estimating protein content in a variety of formulas with different formulations. Downgraded once for serious indirectness.

[11] No studies reported values for infant formula and follow-up formula. The mean conversion factor was therefore derived from dairy sources other than infant formula and follow-up formula (e.g. milk, milk proteins, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration to estimate protein content in a variety of formulas with different formulations. Downgraded once for serious indirectness.

[12] No studies reported values for infant formula and follow-up formula. The mean conversion factor was therefore derived from dairy sources other than infant formula and follow-up formula (e.g. milk, milk proteins, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Downgraded once for serious indirectness.

[13] Fewer than 5 studies contributing to the mean. Downgraded twice for very serious imprecision.

[14] Because this factor was derived from a study using amino acid sequencing, it was started at "high" certainty.

[15] Conversion factors were derived from infant formula and follow-up formula. However, calculation of K´ includes prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids only. Downgraded once for serious indirectness.

[16] Conversion factors were derived from infant formula and follow-up formula. Although K_A 50/50 and 75/25 values are based on setting the ratio of amide to acid arbitrarily at 50/50 or 75/25, it was expected that this would not result in significant enough difference from values derived via direct adjustment. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration in estimating protein content in a variety of formulas with different formulations. There was no indication of reporting error (i.e. selective reporting of K_A values). Not downgraded.

Annex 4 provides information on which studies provided data for each mean conversion factor shown in GRADE evidence profile 1 above.

GRADE evidence profile 2 – Soy-based ingredients

Question: When using the equation *amount of protein (P) = nitrogen to protein conversion factor (K) * amount of nitrogen (N)* to estimate the protein content of soy-based ingredients used in infant formula and follow-up formula, which value of K most closely estimates the true amount of protein (P), where "protein" is defined as amino acid content only?

Population: Infant formula and follow-up formula

Quality assessment							No. of independent measurements	Mean (95% CI)	Certainty	Importance[1]
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations				
Nitrogen to protein conversion factor K´ (unitless) – all soy foods										
12	Total protein and nitrogen[3]	Not serious[4]	Not serious[5]	Very serious[6]	Very serious[7]	None	1	5.71 (single measurement)[2]	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 50/50 (unitless) – all soy foods										
17	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Serious[9]	Not serious[10]	None	28	5.68 (5.66, 5.69)	⨁⨁◯◯ LOW11	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 75/25 (unitless) – all soy foods										
16	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Serious[12]	Not serious[10]	None	26	5.40 (5.38, 5.42)	⨁⨁◯◯ LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with directly calculated amide/acid ratio (unitless) – all soy foods										
19	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Serious[13]	Not serious[10]	None	35	5.65 (5.61, 5.68)	⨁⨁◯◯ LOW	CRITICAL
Nitrogen to protein conversion factor K_B (unitless) – all soy foods										
8	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Serious[14]	Not serious[10]	None	16	5.35 (5.20, 5.51)	⨁⨁◯◯ LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 50/50 (unitless) – formulas only										
2	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Not serious[15]	Very serious[7]	None	4	5.70 (5.69, 5.71)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 75/25 (unitless) – formulas only										
1	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Not serious[15]	Very serious[7]	None	2	5.42 (5.42, 5.42)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor Kv, with directly calculated amide/acid ratio (unitless) – formulas only										
1	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Not serious[16]	Very serious[7]	None	2	5.42 (5.40, 5.44)	⨁◯◯◯ VERY LOW	CRITICAL

CI, confidence interval

[1] Importance for decision-making. In this case, conversion factors are the only "outcome" and thus each of the variations is critical for decision-making.

[2] Regarding K´ and inclusion of prosthetic groups for soy, an additional conversion factor with a value of 5.91 was calculated specifically for the soy 7S protein β-conglycinin (Maubois J-L, Lorient D (2016). Dairy proteins and soy proteins in infant foods nitrogen-to-protein conversion factors. Dairy Sci Tech 96(1):15–25), based on the assumption that all three subunits of β-conglycinin are glycosylated. The authors further use this information to estimate factors for total soy proteins with different 11S/7S ratios, in the range 5.69–5.79. These values were not included in the final analysis as they were not directly measured but estimated, based on assumptions made in reports in the literature.

[3] Because this factor was derived from a study using measurement of crude protein with total nitrogen, it was started at "moderate" certainty.

[4] There was no indication of systematic measurement error or reporting error (i.e. selective reporting of K´ values). Not downgraded.

[5] Inconsistency was not formally assessed as only a single study was available.

[6] No studies reported K´ values for infant formula or follow-up formula. A single study reported K´ for soybeans and this value was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. In addition, calculation of K´ includes prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids only. Downgraded twice for very serious indirectness.

[7] Fewer than 5 studies contributed to the mean. Downgraded twice for very serious imprecision.

[8] Because these factors were derived primarily from studies using amino acid composition analysis, they were started at "moderate" certainty.

[9] Only four values for formulas were identified (from two sources). The mean conversion factor was therefore primarily derived from soy sources other than infant formula and follow-up formula (e.g. soybean, soy isolates, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Although K_A 50/50 and 75/25 values are based on setting the ratio of amide to acid arbitrarily at 50/50 or 75/25, it was expected that this would not result in significant enough difference from values derived via direct adjustment. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration in estimating protein content in a variety of formulas with different formulations. Downgraded once for serious indirectness.

[10] The minimum of 10 measurements is satisfied. Not downgraded.

[11] Although the overall certainty in the evidence for K_A 50/50 and K_A 75/25 was assessed as low, there was greater confidence in the value for K_A 50/50 because it is in line with that of the K_A derived from the directly adjusted amide to acid ratio, which is the most accurate method of assessing amide to acid ratio.

[12] Only two values for formulas were identified (from one source). The mean conversion factor was therefore primarily derived from soy sources other than infant formula and follow-up formula (e.g. soybean, soy isolates, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Although K_A 50/50 and 75/25 values are based on arbitrarily setting the ratio of amide to acid at 50/50 or 75/25, it was expected that this would not result in significant enough difference from values derived via direct adjustment. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration in estimating protein content in a variety of formulas with different formulations. Downgraded once for serious indirectness.

[13] Only two values for formulas were identified (from one source). The mean conversion factor was therefore primarily derived from soy sources other than infant formula and follow-up formula (e.g. soybean, soy isolates, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration in estimating protein content in a variety of formulas with different formulations. Downgraded once for serious indirectness.

[14] No studies reported KB values for infant formula and follow-up formula. The mean conversion factor was therefore derived from soy sources other than infant formula and follow-up formula (e.g. soybean, soy isolates, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Downgraded once for serious indirectness.

[15] Conversion factors were calculated directly from infant formula and follow-up formula. Although K_A 50/50 and 75/25 values are based on setting the ratio of amide to acid arbitrarily at 50/50 or 75/25, it was expected that this would not result in significant enough difference from values derived via direct adjustment. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration in estimating protein content in a variety of formulas with different formulations. Not downgraded.

[16] Conversion factors were calculated directly from infant formula and follow-up formula. Also, unlike K_B, K_A values do not explicitly take into consideration non-protein nitrogen; however, non-protein nitrogen can vary considerably across food samples. It is therefore difficult to assess the level of indirectness in using a conversion factor that does not take non-protein nitrogen into consideration in estimating protein content in a variety of formulas with different formulations. Not downgraded.

Annex 5 provides information on which studies provided data for each mean conversion factor shown in GRADE evidence profile 2 above.

GRADE evidence profile 3 – Dairy-based ingredients

Question: When using the equation *amount of protein (P) = nitrogen to protein conversion factor (K) * amount of nitrogen (N)* to estimate the protein content of dairy-based ingredients used in infant formula and follow-up formula, which value of K most closely estimates the true amount of protein (P), where "protein" is defined as amino acid content plus prosthetic groups?

Population: Infant formula and follow-up formula

Quality assessment							No. of independent measurements	Mean (95% CI)	Certainty	Importance[1]
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations				
Nitrogen to protein conversion factor K´ (unitless) – all dairy foods										
5	Mixed[2]	Not serious[3]	Not serious[4]	Serious[5]	Not serious[6]	None	10	6.32 (6.26, 6.38)	⨁⨁⨁◯ MODERATE	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 50/50 (unitless) – all dairy foods										
13	Amino acid analysis[7]	Not serious[4]	Not serious[5]	Serious[8]	Not serious[7]	None	31	6.06 (6.00, 6.12)	⨁⨁◯◯ LOW[9]	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 75/25 (unitless) – all dairy foods										
12	Amino acid analysis[7]	Not serious[4]	Not serious[5]	Very serious[10]	Not serious[7]	None	14	5.83 (5.77, 5.89)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with directly calculated amide/acid ratio (unitless) – all dairy foods										
12	Amino acid analysis[7]	Not serious[4]	Not serious[5]	Very serious[10]	Not serious[7]	None	16	6.03 (5.98, 6.07)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_B (unitless) – all dairy foods										
3	Amino acid analysis[7]	Not serious[4]	Not serious[5]	Very serious[10]	Very serious[11]	None	3	5.55 (5.31, 5.78)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K´ (unitless) – formulas only										
1	Amino acid sequencing[12]	Not serious[3]	Not serious[4]	Not serious[13]	Very serious[11]	None	1	6.38 (single measurement)	⨁⨁◯◯ LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 50/50 (unitless) – formulas only										
1	Amino acid analysis[7]	Not serious[3]	Not serious[4]	Serious[14]	Not serious[6]	None	11	6.08 (6.05, 6.12)	⨁⨁◯◯ LOW	CRITICAL

CI, confidence interval

[1] Importance for decision-making. In this case, conversion factors are the only "outcome" and thus each of the variations is critical for decision-making.

[2] Amino acid sequencing and measurement (weighing) of crude protein with total nitrogen analysis. Because this factor was derived primarily from studies using amino acid sequencing, it was started at "high" certainty.

[3] There was no indication of systematic measurement error or reporting error (i.e. selective reporting of K´ values). Not downgraded.

[4] Inconsistency was assessed by considering the level of variance around the mean. The 95% CI suggests very little variation around the mean. Not downgraded.

[5] Only a single K´ value for infant formula and follow-up formula was identified in the literature review. The mean conversion factor was therefore primarily derived from dairy sources other than infant formula and follow-up formula (e.g. milk, milk proteins, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Downgraded once for serious indirectness.

[6] The minimum of 10 measurements is satisfied. Not downgraded.

[7] Because these factors were derived primarily from studies using amino acid composition analysis they were started at "moderate" certainty.

[8] Of the 31 measurements, 11 come from infant formula and follow-up formula. Although K_A 50/50 and 75/25 values are based on arbitrarily setting the ratio of amide to acid at 50/50 or 75/25, it was expected that this would not result in significant enough difference from values derived via direct adjustment. Also, calculation of K_A does not include prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids plus prosthetic groups. Downgraded once for serious indirectness.

[9] In addition to having greater confidence in the K_A 50/50 value relative to K_A 75/25 because the former includes a significant number of studies which derived conversion factors directly from infant formula and follow-up formula (and therefore was not downgraded for serious indirectness), there was greater confidence in this value because it is in line with that of the K_A derived from the directly adjusted amide to acid ratio, which is the most accurate method of assessing amide to acid ratio.

[10] No studies reported K_A or K_B values for infant formula and follow-up formula. The mean conversion factor was therefore derived from dairy sources other than infant formula and follow-up formula (e.g. milk, milk proteins, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Also, calculation of K_A or K_B does not include prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids plus prosthetic groups. Downgraded twice for very serious indirectness.

[11] Fewer than 5 studies contributed to the mean. Downgraded twice for very serious imprecision.

[12] Because this factor was derived from a study using amino acid sequencing, it was started at "high" certainty.

[13] Conversion factors were derived from infant formula and follow-up formula. Not downgraded.

[14] Conversion factors were derived from infant formula and follow-up formula. However, calculation of K_A does not include prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids plus prosthetic groups. Downgraded once for serious indirectness.

Annex 4 provides information on which studies provided data for each mean conversion factor shown in GRADE evidence profile 3 above.

GRADE evidence profile 4 – Soy-based ingredients

Question: When using the equation *amount of protein (P) = nitrogen to protein conversion factor (K) * amount of nitrogen (N)* to estimate the protein content of soy-based ingredients used in infant formula and follow-up formula, which value of K most closely estimates the true amount of protein (P), where "protein" is defined as amino acid content plus prosthetic groups?

Population: Infant formula and follow-up formula

Quality assessment							No. of independent measurements	Mean (95% CI)	Certainty	Importance[1]
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations				
Nitrogen to protein conversion factor K´ (unitless) – all soy foods										
12	Total protein and nitrogen[3]	Not serious[4]	Not serious[5]	Serious[6]	Very serious[7]	None	1	5.71 (single measurement)[2]	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 50/50 (unitless) – all soy foods										
17	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Very serious[9]	Not serious[10]	None	28	5.68 (5.66, 5.69)	⨁◯◯◯ VERY LOW[11]	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 75/25 (unitless) – all soy foods										
16	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Very serious[12]	Not serious[10]	None	26	5.40 (5.38, 5.42)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with directly calculated amide/acid ratio (unitless) – all soy foods										
19	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Very serious[12]	Not serious[10]	None	35	5.65 (5.61, 5.68)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_B (unitless) – all soy foods										
8	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Very serious[13]	Not serious[10]	None	16	5.35 (5.20, 5.51)	⨁◯◯◯O VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 50/50 (unitless) – formulas only										
2	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Serious[14]	Very serious[8]	None	4	5.70 (5.69, 5.71)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with amide/acid ratio of 75/25 (unitless) – formulas only										
1	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Serious[14]	Very serious[8]	None	2	5.42 (5.42, 5.42)	⨁◯◯◯ VERY LOW	CRITICAL
Nitrogen to protein conversion factor K_A, with directly calculated amide/acid ratio (unitless) – formulas only										
1	Amino acid analysis[8]	Not serious[4]	Not serious[5]	Serious[14]	Very serious[8]	None	2	5.42 (5.40, 5.44)	⨁◯◯◯ VERY LOW	CRITICAL

CI, confidence interval

[1] Importance for decision-making. In this case, conversion factors are the only "outcome" and thus each of the variations is critical for decision-making.

[2] Regarding K´ and inclusion of prosthetic groups for soy, an additional conversion factor with a value of 5.91 was calculated specifically for the soy 7S protein β-conglycinin (Maubois J-L, Lorient D (2016). Dairy proteins and soy proteins in infant foods nitrogen-to-protein conversion factors. Dairy Sci Tech 96(1):15–25), based on the assumption that all three subunits of β-conglycinin are glycosylated. The authors further use this information to estimate factors for total soy proteins with different 11S/7S ratios, in the range 5.69–5.79. These values were not included in the final analysis as they were not directly measured but estimated, based on assumptions made in reports in the literature.

[3] Because this factor was derived from a study using measurement of crude protein with total nitrogen, it was started at "moderate" certainty.

[4] There was no indication of systematic measurement error or reporting error (i.e. selective reporting of K´ values). Not downgraded.

[5] Inconsistency was not formally assessed as only a single study was available.

[6] No studies reported K´ values for infant formula or follow-up formula. A single study reported K´ for soybeans and this value was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Downgraded once for serious indirectness.

[7] Fewer than 5 studies contributed to the mean. Downgraded twice for very serious imprecision.

[8] Because these factors were derived primarily from studies using amino acid composition analysis, they were started at "moderate" certainty.

[9] Only four values for formulas were identified (from two sources). The mean conversion factor was therefore derived from soy sources other than infant formula and follow-up formula (e.g. soybean, soy isolates, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Also, calculation of K_A or K_B does not include prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids plus prosthetic groups. Downgraded twice for very serious indirectness.

[10] The minimum of 10 measurements is satisfied. Not downgraded.

[11] Although the overall certainty in the evidence for K_A 50/50 and K_A 75/25 was assessed as very low, there was greater confidence in the value for K_A 50/50 because it is in line with that of the K_A derived from the directly adjusted amide to acid ratio, which is the most accurate method of assessing amide to acid ratio.

[12] Only two values for formulas were identified (from one source). The mean conversion factor was therefore derived from soy sources other than infant formula and follow-up formula (e.g. soybean, soy isolates, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Also, calculation of K_A or K_B does not include prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids plus prosthetic groups. Downgraded twice for very serious indirectness.

[13] No studies reported KB values for infant formula or follow-up formula. The mean conversion factor was therefore derived from soy sources other than infant formula and follow-up formula (e.g. soybean, soy isolates, etc.) and was thus considered an indirect assessment of the conversion factor for infant formula and follow-up formula. Also, calculation of K_A or K_B does not include prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids plus prosthetic groups. Downgraded twice for very serious indirectness.

[14] Conversion factors were calculated directly from infant formula or follow-up formula. However, calculation of K_A or K_B does not include prosthetic groups in the protein mass and the PICO question specifically defines protein as amino acids plus prosthetic groups. Downgraded once for serious indirectness.

Annex 5 provides information on which studies provided data for each mean conversion factor shown in GRADE evidence profile 4 above.

ANNEX 4

STUDIES INCLUDED IN THE GRADE ASSESSMENT: DAIRY

The studies (data sets) listed below are those used to derive the mean conversion factors found in each row of GRADE evidence profiles 1 and 3.

ROW	# OF STUDIES	STUDIES	
ALL DAIRY ITEMS			
1. K´	5	Jones, 1931 Van Boekel M, 1987 Hammersten, 1883	Maubois and Lorient, 2016 Rouch et al., 2008
2. K_A (50/50)	13	Boisen et al., 1987 Ceballos et al., 2009 Csapo-Kiss et al., 1994 Derham, 1982 Featherston et al., 1964 Japan Vegetable Food Association[a] Krizova et al., 2013	Marino et al., 2010 Maubois and Lorient, 2016 Rutherfurd and Moughan, 1998 Sarwar et al., 1983b Tomotake et al., 2001 Zándoki, 2006
3. K_A (75/25)	12	Boisen et al., 1987 Ceballos et al., 2009 Csapo-Kiss et al., 1994 Derham, 1982 Featherston et al., 1964 Japan Vegetable Food Association[a]	Krizova et al., 2013 Marino et al., 2010 Rutherfurd and Moughan, 1998 Sarwar et al., 1983b Tomotake et al., 2001 Zándoki, 2006
4. K_A (direct)	12	Boisen et al., 1987 Ceballos et al., 2009 Csapo-Kiss et al., 1994 Featherston et al., 1964 Japan Vegetable Food Association[a] Krizova et al., 2013	Marino et al., 2010 Rutherfurd and Moughan, 1998 Sarwar et al., 1983b Sosulski, 1990 Tomotake et al., 2001 Zándoki, 2006
5. K_B	3	Boisen et al., 1987 Fujihara et al., 2010 Salo-väänänen and Koivistoinen, 1996	
FORMULAS ONLY			
6. K´	1	Maubois and Lorient, 2016	
7. K_A (50/50)	1	Nestec[a]	

[a] data submitted during call for data

ANNEX 5

STUDIES INCLUDED IN THE GRADE ASSESSMENT: SOY

The studies (data sets) listed below are those used to derive the mean conversion factors found in each row of GRADE evidence profiles 2 and 4

ROW	# OF STUDIES	STUDIES	
ALL SOY ITEMS			
1. K´	1	Jones, 1931	
2. K_A (50/50)	17	Boisen et al., 1987 Derham, 1982 Dupont[a] ENSA[a] Erasmus et al., 1994 FAO/WHO, 2016a Gorissen et al., 2018 Health Canada[a] Hughes et al., 2011	Japan Vegetable Food Association[a] Morr, 1981, 1982 Nestec[a] Rutherfurd and Moughan, 1998 Sosulski and Holt, 1980 Sriperm et al., 2011 Tomotake et al., 2001 Zarkadas et al., 1997
3. K_A (75/25)	16	Boisen et al., 1987 Derham, 1982 Dupont[a] ENSA[a] Erasmus et al., 1994 FAO/WHO, 2016a Gorissen et al., 2018 Health Canada[a]	Hughes et al., 2011 Japan Vegetable Food Association[a] Morr, 1981, 1982 Rutherfurd and Moughan, 1998 Sosulski and Holt, 1980 Sriperm et al., 2011 Tomotake et al., 2001 Zarkadas et al., 1997
4. K_A (direct)	19	Boisen et al., 1987 Dupont[a] ENSA[a] Erasmus et al., 1994 FAO/WHO, 2016a Gorissen et al., 2018 Health Canada[a] Hughes et al., 2011 Japan Vegetable Food Association* Morr, 1981, 1982	Mossé, 1990 Rutherfurd and Moughan, 1998 Sarwar et al., 1973 Sosulski and Holt, 1980 Sosulski and Sarwar, 1973 Tkachuk, 1969 Sriperm et al., 2011 Tomotake et al., 2001 Zarkadas et al., 1997
5. K_B	8	Boisen et al., 1987 ENSA[a] Fujihara et al., 2010 Japan Vegetable Food Association*	Morr, 1981, 1982 Mossé, 1990 Sosulski and Holt, 1980 Sriperm et al., 2011
FORMULAS ONLY			
6. K_A (50/50)*	2	Health Canada[a] Nestec[a]	
7. K_A (75/25)*	1	Nestec[a]	
8. K_A (direct)*	1	Nestec[a]	

[a] data submitted during call for data

BIBLIOGRAPHY

Adeola O, Buchanansmith JG, Early RJ (1988). Gas and high-performance liquid-chromatographic analyses of amino acids in soybean meal, corn and tricale. J Food Biochem. 12(3):171–81 doi:10.1111/j.1745-4514.1988.tb00370.x

(https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1745-4514.1988.tb00370.x).

Adesina K (2012). Effect of breed on the composition of cow milk under traditional management practices in Ado-Ekiti, Nigeria. J Appl Sci Environ Manage. 16(1):55–9

(https://www.ajol.info/index.php/jasem/article/view/90752/80185).

Akhlaghi Y, Ghaffari S, Attar H, Alamir Hoor A (2015). A rapid hydrolysis method and DABS-Cl derivatization for complete amino acid analysis of octreotide acetate by reversed phase HPLC. Amino Acids. 47(11):2255–63 doi:10.1007/s00726-015-1999-9

(https://link.springer.com/article/10.1007%2Fs00726-015-1999-9).

Alpert AJ (1990). Hydrophilic-interaction chromatography for the separation of peptides, nucleic acids and other polar compounds. J Chromatogr. 499:177–96

(https://www.sciencedirect.com/science/article/pii/S0021967300969723).

Andree S, Schwaegele F, Martin D, Ostermeier U, Rehbein H (2012). Determining metabolic profiles of food of animal origin. Part. 1. Free amino acids. Fleischwirtschaft. 92(5):103–7

(https://search.ebscohost.com/login.aspx?direct=true&db=ffh&AN=2012-09-Aj4967&lang=fr&site=ehost-live).

Angell AR, Mata L, de Nys R, Paul NA (2016). The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. J Appl Phycol. 28(1):511–24 doi:10.1007/s10811-015-0650-1

(https://link.springer.com/article/10.1007%2Fs10811-015-0650-1).

Antonio Agustí J, Pío Beltrán J (1982). Quantitative determination of the protein content of citrus leaf extracts: a comparative study. Anal Biochem. 127(2):368–71

(https://www.sciencedirect.com/science/article/pii/0003269782901889).

Aoyama C, Santa T, Tsunoda M, Fukushima T, Kitada C, Imai K (2004). A fully automated amino acid analyzer using NBD-F as a fluorescent derivatization reagent. Biomed Chromatogr. 18(9):630–6 doi:10.1002/bmc.365

(https://onlinelibrary.wiley.com/doi/pdf/10.1002/bmc.365).

Ayers JS, Coolbear KP, Elgar DF, Pritchard M (1998). Process for isolating glycomacropeptide from dairy products with a phenylalanine impurity of 0.5% w/w. NZ 96-299483 [19961001]

(http://www.freepatentsonline.com/6555659.html).

Bailon-Perez MI, Garcia-Campana AM, Cruces-Blanco C, Del Olmo Iruela M (2007). Large-volume sample stacking for the analysis of seven beta-lactam antibiotics in milk samples of different origins by CZE. Electrophoresis. 28(22):4082–90 doi:10.1002/elps.200700305

(https://www.ncbi.nlm.nih.gov/pubmed/17960857).

Bainor A, Chang L, McQuade TJ, Webb B, Gestwicki JE (2011). Bicinchoninic Acid (BCA) assay in low volume. Anal Biochem. 410(2):310–2

(https://www.sciencedirect.com/science/article/pii/S0003269710007281).

Barbano DM, Lynch JM (2006). Major advances in testing of dairy products: milk component and dairy product attribute testing. J Dairy Sci. 89(4):1189–94 doi:10.3168/jds.S0022-0302(06)72188-9

(https://www.sciencedirect.com/science/article/pii/S0022030206721889?via%3Dihub).

Beavers HC, Ockeriwan HW, Cahill VR, Parrett NA, Borton RJ (1973). Ultraviolet spectrophotometric determination of protein in meat and meat products. J Food Sci. 38(6):1087–8 doi:10.1111/j.1365-2621.1973.tb02159.x

(https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2621.1973.tb02159.x).

Bech-Andersen S (1991). Determination of tryptophan with HPLC after alkaline-hydrolysis in autoclave using alpha-methyl-tryptophan as internal standard. Acta Agric Scand. 41(3):305–9 doi:10.1080/00015129109439913

(https://www.tandfonline.com/doi/abs/10.1080/00015129109439913).

Beljkaš B, Matić J, Milovanović I, Jovanov P, Mišan A, Saric L (2010). Rapid method for determination of protein content in cereals and oilseeds: Validation, measurement uncertainty and comparison with the Kjeldahl method. Accred Qual Assur. 15:555–61 doi:10.1007/s00769-010-0677-6

(https://link.springer.com/article/10.1007/s00769-010-0677-6).

Benson JR, Hare PE (1975). O-phthalaldehyde: fluorogenic detection of primary amines in the picomole range. Comparison with fluorescamine and ninhydrin. Proc Natl Acad Sci. 72(2):619–22

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC432365/).

Benton Jones Jr. J (1987). Kjeldahl nitrogen determination – what's in a name. J Plant Nutr. 10(9–16):1675–82

(https://www.tandfonline.com/doi/abs/10.1080/01904168709363706).

Benton Jones Jr. J (1991). Kjeldahl method for nitrogen determination. Athens, GA: Micro-Macro Publishing, Inc.

Berthelot MPE (1859). Violet D'aniline. Repert Chim Appl. 1:284.

Biancarosa I, Espe M, Bruckner CG, Heesch S, Liland N, Waagbo R et al. (2017). Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. J Appl Phycol. 29(2):1001–9 doi:10.1007/s10811-016-0984-3

(https://link.springer.com/article/10.1007%2Fs10811-016-0984-3).

Bidlingmeyer BA, Cohen SA, Tarvin TL (1984). Rapid analysis of amino acids using pre-column derivatization. J Chromatogr. 336(1):93–104

(https://www.sciencedirect.com/science/article/pii/S0378434700851336).

Bidlingmeyer BA, Cohen SA, Tarvin TL, Frost B (1987). A new, rapid, high-sensitivity analysis of amino acids in food type samples. J Assoc Off Anal Chem. 70(2):241–7

(https://www.ncbi.nlm.nih.gov/pubmed/3571118).

Bjarnadottir M, Adalbjornsson BV, Nilsson A, Slizyte R, Roleda MY, Hreggvidsson GO et al. (2018). Palmaria palmata as an alternative protein source: enzymatic protein extraction, amino acid composition, and nitrogen-to-protein conversion factor. J Appl Phycol. 30(3):2061–70 doi:10.1007/s10811-017-1351-8

(https://link.springer.com/article/10.1007%2Fs10811-017-1351-8).

Blatný P, Kvasnicka F (1999). Application of capillary isotachophoresis and capillary zone electrophoresis to the determination of inorganic ions in food and feed samples. J Chromatogr A. 834(1-2):419–31 doi:10.1016/s0021-9673(98)00905-4

(https://europepmc.org/abstract/MED/10189697).

Boisen S, Bech-Andersen S, Eggum BrO (1987). A critical view on the conversion factor 6.25 from total nitrogen to protein. Acta Agric Scand. 37(3):299–304 doi:10.1080/00015128709436560

(https://doi.org/10.1080/00015128709436560).

Bonde M, Pontoppidan H, Pepper DS (1992). Direct dye binding – A quantitative assay for solid-phase immobilized protein. Anal Biochem. 200(1):195–8

(https://www.sciencedirect.com/science/article/pii/000326979290298L).

Bonfatti V, Di Martino G, Carnier P (2011). Effectiveness of mid-infrared spectroscopy for the prediction of detailed protein composition and contents of protein genetic variants of individual milk of Simmental cows. J Dairy Sci. 94(12):5776–85

(https://www.sciencedirect.com/science/article/pii/S0022030211006138).

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72(1):248–54

(https://www.sciencedirect.com/science/article/pii/0003269776905273).

Bradstreet RB (1954). The Kjeldahl method for organic nitrogen. Anal Chem. 26(1):185–7

(https://www.sciencedirect.com/book/9781483232980/the-kjeldahl-method-for-organic-nitrogen).

Briend-Marchal A, Medaille C, Braun JP (2005). Comparison of total protein measurement by Biuret method and refractometry in canine and feline plama. Rev Med Vet (Toulouse). 156(12):615–9

(https://www.revmedvet.com/2005/RMV156_615_619.pdf).

Brimer CM, Murray-McIntosh RP, Neale TJ, Davis PF (1995). Nonenzymatic glycation interferes with protein concentration determinations. Anal Biochem. 224(1):461–3

(https://www.sciencedirect.com/science/article/pii/S0003269785710743).

Brooke O, Wood C, Barley J (1982). Energy balance, nitrogen balance, and growth in preterm infants fed expressed breast milk, a premature infant formula, and two low-solute adapted formulae. Arch Dis Child. 57(12):898–904.

Buszewski B, Noga S (2012). Hydrophilic interaction liquid chromatography (HILIC) – A powerful separation technique. Anal Bioanal Chem. 402(1):231–47 doi:10.1007/s00216-011-5308-5

(https://www.ncbi.nlm.nih.gov/pubmed/21879300).

Callejon RM, Troncoso AM, Morales ML (2010). Determination of amino acids in grape-derived products: a review. Talanta. 81(4–5):1143–52 doi:10.1016/j.talanta.2010.02.040

(https://www.sciencedirect.com/science/article/pii/S0039914010001372?via%3Dihub).

Carpenter KJ, Bjarnason J (1968). Nutritional evaluation of proteins by chemical methods. In: Bender AE, Kihlberg R, Löfqvist B, Munck L, editors. Evaluation of Novel Protein Products. Oxford: Pergamon Press, 161.

Carraway KL, Koshland DE (1972). Carbodiimide modification of proteins. Methods Enzymol. 25:616–23

(https://www.sciencedirect.com/science/article/pii/S0076687972250601).

Castro-Puyana M, Garcia-Canas V, Simo C, Cifuentes A (2012). Recent advances in the application of capillary electromigration methods for food analysis and Foodomics. Electrophoresis. 33(1):147–67 doi:10.1002/elps.201100385

(https://www.ncbi.nlm.nih.gov/pubmed/22147337).

Castro ER, Manz A (2015). Present state of microchip electrophoresis: state of the art and routine applications. J Chromatogr A. 1382:66–85 doi:10.1016/j.chroma.2014.11.034

(https://www.sciencedirect.com/science/article/pii/S0021967314017920?via%3Dihub).

Ceballos LS, Morales ER, Martinez LP, Extremera FG, Sampelayo MR (2009). Utilization of nitrogen and energy from diets containing protein and fat derived from either goat milk or cow milk. J Dairy Res. 76(4):497–504 doi:10.1017/S0022029909990252

(https://www.ncbi.nlm.nih.gov/pubmed/19825215).

Chang KC, Skauge LH, Satterlee LD (1989). Analysis of amino acids in soy isolates and navy beans using precolumn derivatization with phenylisothiocyanate and reversed-phase high performance liquid chromatography. J Food Sci. 54(3):756 doi:10.1111/j.1365-2621.1989.tb04699.x

(https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2621.1989.tb04699.x).

Chebaro Y, Lorenz M, Fa A, Zheng R, Gustin M (2017). Adaptation of candida albicans to reactive sulfur species. Genetics. 206(1):151–62 doi:10.1534/genetics.116.199679

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5419466/pdf/151.pdf).

Chen Q, Klemm N, Duncan G, Jeng I (1990). Sensitive Benedict test. Analyst. 115(1):109–10 doi:10.1039/AN9901500109

(https://pubs.rsc.org/en/content/articlelanding/1990/AN/an9901500109#!divAbstract).

Chen X, Zhao G, Zhang Y, Han L, Xiao W (2017). Nitrogen-to-protein conversion factors for crop residues and animal manure common in China. J Agric Food Chem. 65(42):9186–90 doi:10.1021/acs.jafc.7b03441

(https://doi.org/10.1021/acs.jafc.7b03441).

Chen ZL, Warren CR, Adams MA (2000). Separation of amino acids in plant tissue extracts by capillary zone electrophoresis with indirect UV detection using aromatic carboxylates as background electrolytes. Chromatographia. 51(3):180–6 doi:10.1007/bf02490562

(https://doi.org/10.1007/BF02490562).

Chiappelli F, Vasil A, Haggerty DF (1979). The protein concentration of crude cell and tissue extracts as estimated by the method of dye binding: comparison with the Lowry method. Anal Biochem. 94(1):160–5

(https://www.sciencedirect.com/science/article/pii/0003269779908054).

Cohen SA, Michaud DP (1993). Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. Anal Biochem. 211(2):279–87 doi:10.1006/abio.1993.1270

(https://www.sciencedirect.com/science/article/pii/S0003269783712704?via%3Dihub).

Compton SJ, Jones CG (1985). Mechanism of dye response and interference in the Bradford protein assay. Anal Biochem. 151(2):369–74

(https://www.sciencedirect.com/science/article/pii/0003269785901903?via%3Dihub).

Csapo-Kiss Z, Stefler J, Martin TG, Makray S, Csapo J (1994). Protein content, amino acid composition, biological value and micro- and macroelement content of mare's milk. Acta Alimentaria. 23(2):179–94.

Culbertson CT, Jacobson SC, Ramsey JM (2000). Microchip devices for high-efficiency separations. Anal Chem. 72(23):5814–9 doi:10.1021/ac0006268

(https://doi.org/10.1021/ac0006268).

Cuq JL, Besancon P, Chartier L, Cheftel C (1978). Oxidation of methionine residues of food proteins and nutritional availability of protein-bound methionine sulphoxide. Food Chem. 3(2):85–102 doi:10.1016/0308-8146(78)90027-4

(https://www.sciencedirect.com/science/article/pii/0308814678900274?via%3Dihub).

Dai Z, Wu Z, Jia S, Wu G (2014). Analysis of amino acid composition in proteins of animal tissues and foods as pre-column o-phthaldialdehyde derivatives by HPLC with fluorescence detection. J Chromatogr B. 964:116–27

(https://www.sciencedirect.com/science/article/pii/S1570023214002153).

Darragh AJ, Moughan PJ (2005). The effect of hydrolysis time on amino acid analysis. J AOAC Int. 88(3):888–93.

de Castro RJS, Sato HH (2015). Biologically active peptides: processes for their generation, purification and identification and applications as natural additives in the food and pharmaceutical industries. Food Res Int. 74:185–98

(https://www.sciencedirect.com/science/article/pii/S0963996915300028).

de Marchi M, Toffanin V, Cassandro M, Penasa M (2014). Invited review: mid-infrared spectroscopy as phenotyping tool for milk traits. J Dairy Sci. 97(3):1171–86

(https://www.sciencedirect.com/science/article/pii/S0022030214000253).

de Oliveira MAL, Porto BLS, Bastos CdA, Sabarense CM, Vaz FAS, Neves LNO et al. (2016). Analysis of amino acids, proteins, carbohydrates and lipids in food by capillary electromigration methods: a review. Anal Meth. 8(18):3649–80 doi:10.1039/C5AY02736E

(https://pubs.rsc.org/en/Content/ArticleLanding/2016/AY/C5AY02736E).

Derham O (1982). Nitrogen-content of proteins and the protein nitrogen multiplication factor. LWT – Food Sci Tech. 15(4):226–31.

Desrosiers T, Savoie L, Bergeron G, Parent G (1989). Estimation of lysine damage in heated whey proteins by furosine determinations in conjunction with the digestion cell technique. J Agric Food Chem. 37:1385–91

(https://pubs.acs.org/doi/abs/10.1021/jf00089a039).

DeVries JW, Greene GW, Payne A, Zbylut S, Scholl PF, Wehling P et al. (2017). Non-protein nitrogen determination: a screening tool for nitrogenous compound adulteration of milk powder. Int Dairy J. 68:46–51

(https://www.sciencedirect.com/science/article/pii/S0958694616303582).

Diniz GS, Barbarino E, Oiano-Neto J, Pacheco S, Lourenço SO (2011). Gross chemical profile and calculation of nitrogen-to-protein conversion factors for five tropical seaweeds. Am J Plant Sci. 2(3):287–96 doi:10.4236/ajps.2011.23032

(https://www.scirp.org/journal/PaperInformation.aspx?paperID=7572#abstract).

Dong M, Qin L, Xue J, Du M, Lin SY, Xu XB et al. (2018). Simultaneous quantification of free amino acids and 5'-nucleotides in shiitake mushrooms by stable isotope labeling-LC-MS/MS analysis. Food Chem. 268:57–65 doi:10.1016/j.foodchem.2018.06.054

(https://www.ncbi.nlm.nih.gov/pubmed/30064799).

Doumas BT, Bayse D, Borner K, Carter R, Elevitch F, Garber C et al. (1981a). A candidate reference method for determination of total protein in serum. II. Test for transferability. Clin Chem. 27(10):1651–4

(https://www.ncbi.nlm.nih.gov/pubmed/7285315).

Doumas BT, Bayse DD, Carter RJ, Peters T, Schaffer R (1981b). A candidate reference method for determination of total protein in serum. I. Development and validation. Clin Chem. 27(10):1642–50

(http://clinchem.aaccjnls.org/content/27/10/1642.short).

Dumas JBA (1831). Procedes de l'analyse organique. Ann Chim Phys. 2(47):198.

Dyer B (1895). Kjeldahl's method for the determination of nitrogen. J Chem Soc Trans. 67:811–7.

Eckfeldt JH, Kershaw MJ, Dahl, II (1984). Direct analysis for urinary protein with Biuret reagent, with use of urine ultrafiltrateblanking: comparison with a manual Biuret method involving trichloroaceticacid precipitation. Clin Chem. 30(3):443–6

(http://clinchem.aaccjnls.org/content/clinchem/30/3/443.full.pdf).

Einarsson S, Josefsson B, Lagerkvist S (1983). Determination of amino acids with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography. J Chromatogr A. 282:609–18

(https://www.sciencedirect.com/science/article/pii/S0021967300916388).

El Deeb S, Iriban MA, Gust R (2011). MEKC as a powerful growing analytical technique. Electrophoresis. 32(1):166–83 doi:10.1002/elps.201000398

(https://www.ncbi.nlm.nih.gov/pubmed/21171121).

Elias RJ, McClements J, Decker EA (2005). Antioxidant activity of cysteine, tryptophan, and methionine residues in continuous phase β-lactoglobulin in oil-in-water emulsions. J Agric Food Chem. 53:10248–53

(https://pubs.acs.org/doi/10.1021/jf0521698).

Ellinger GM, Duncan A (1976). The determination of methionine in proteins by gas-liquid chromatography. Biochem J. 155:615–21

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1172884/pdf/biochemj00535-0170.pdf).

Erasmus LJ, Botha PM, Cruywagen CW, Meissner HH (1994). Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feedstuffs. J Dairy Sci. 77(2):541–51 doi:10.3168/jds.S0022-0302(94)76982-4

(https://www.sciencedirect.com/science/article/pii/S0022030294769824?via%3Dihub).

Erny GL, León C, Marina ML, Cifuentes A (2008). Time of flight versus ion trap MS coupled to CE to analyse intact proteins. J Sep Sci. 31(10):1810–8 doi:10.1002/jssc.200700651

(https://onlinelibrary.wiley.com/doi/abs/10.1002/jssc.200700651).

Etzion Y, Linker R, Cogan U, Shmulevich I (2004). Determination of protein concentration in raw milk by mid-infrared Fourier transform infrared/attenuated total reflectance spectroscopy. J Dairy Sci. 87(9):2779–88

(https://www.sciencedirect.com/science/article/pii/S0022030204734050).

Ezeagu IE, Petzke JK, Metges CC, Akinsoyinu AO, Ologhobo AD (2002). Seed protein contents and nitrogen-to-protein conversion factors for some uncultivated tropical plant seeds. Food Chem. 78(1):105–9 doi:10.1016/S0308-8146(02)00105-X

(https://www.sciencedirect.com/science/article/pii/S030881460200105X?via%3Dihub).

FAO/WHO (1981). Standard for infant formula and formulas for special medical purposes intended for infants. Geneva: Food and Agriculture Organization (FAO)/World Health Organization(WHO)

(http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B72-1981%252FCXS_072e.pdf).

FAO/WHO (1987). Standard for follow-up formula. Geneva: Food and Agriculture Organization(FAO)/World Health Organization(WHO), CXS 156-1987

(http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B156-1987%252FCXS_156e.pdf).

FAO/WHO (2012). Terms of reference and rules of procedure for the FAO/WHO Joint Expert Meetings on Nutrition (JEMNU). Geneva: Food and Agriculture Organization(FAO)/World Health Organization(WHO)

(http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-720-38%252FTORs_JEMNU.pdf).

FAO/WHO (2016a). Codex committee on methods of analysis sampling. Agenda Item 2 MAS/37 CRD/7, Budapest, Hungary: Joint FAO/WHO Food Standard Programme, Thirty-seventhth Session

(http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-715-37%252FCRD%252Fma37_CRD6x.pdf).

FAO/WHO (2016b). Joint FAO/WHO Food Standard Programme – Report of the 37th Session of the CCMAS, CX/MAS REP16/MAS. Geneva: Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO).

Featherston WR, Frazeur DR, Hill DL, Noller CH, Parmelee CE (1964). Constancy of amino acid composition of cow's milk protein under changing ration. J Dairy Sci. 47(12):1417–8 doi:10.3168/jds.S0022-0302(64)88931-1

(https://doi.org/10.3168/jds.S0022-0302(64)88931-1).

Fekkes D (1996). State-of-the-art of high-performance liquid chromatographic analysis of amino acids in physiological samples. J Chromatogr B Biomed Appl. 682(1):3–22

(https://www.ncbi.nlm.nih.gov/pubmed/8832421).

Ferreira DS, Galão OF, Pallone JAL, Poppi RJ (2014). Comparison and application of near-infrared (NIR) and mid-infrared (MIR) spectroscopy for determination of quality parameters in soybean samples. Food Control. 35(1):227–32

(https://www.sciencedirect.com/science/article/pii/S0956713513003496).

Finete VdLM, Gouvêa MM, Marques FFdC, Netto ADP (2013). Is it possible to screen for milk or whey protein adulteration with melamine, urea and ammonium sulphate, combining Kjeldahl and classical spectrophotometric methods? Food Chem. 141(4):3649–55

(https://www.sciencedirect.com/science/article/pii/S0308814613008236).

Finley JW (1985). Reducing variability in amino acid analysis. St Paul, Mn: American Association of Cereal Chemists.

Folin O, Ciocalteu V (1927). On tyrosine and tryptophane determinations in proteins. J Biol Chem. 73:627–50

(http://www.jbc.org/content/73/2/627.full.pdf).

Fountoulakis M, Juranville J-F, Manneberg M (1992). Comparison of the Coomassie brilliant blue, bicinchoninic acid and Lowry quantitation assays, using non-glycosylated and glycosylated proteins. J Biochem Bioph Meth. 24(3):265–74

(https://www.sciencedirect.com/science/article/pii/0165022X94900787).

Fraenkel-Conrat H, Cooper M (1944). The use of dye for the determination of acid and basic groups in proteins. J Biol Chem. 154(1):239–46

(http://www.jbc.org/content/154/1/239.full.pdf).

Friedman M, Pang J, Smith GA (1984). Ninhydrin-reactive lysine in food proteins. J Food Sci. 49:10–20

(https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2621.1984.tb13656.x).

Fujihara S, Sasaki H, Aoyagi Y, Sugahara T (2008). Nitrogen-to-protein conversion factors for some cereal products in Japan. J Food Sci. 73(3):C204–9 doi:10.1111/j.1750-3841.2008.00665.x

(https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1750-3841.2008.00665.x).

Fujihara S, Sasaki H, Sugahara T (2010). Nitrogen-to-protein conversion factors for some pulses and soybean products. J Integrated Study of Dietary Habits. 21(1):60–6 doi:10.2740/jisdh.21.60

(https://www.jstage.jst.go.jp/article/jisdh/21/1/21_1_60/_pdf).

Gadsden RH (1983). Comparison of gel filtration-Biuret, Coomassie Blue, and turbidimetric methods for urine total protein. Ann Clin Lab Sci. 13(4):351–2.

Garcia-Canas V, Simo C, Castro-Puyana M, Cifuentes A (2014). Recent advances in the application of capillary electromigration methods for food analysis and Foodomics. Electrophoresis. 35(1):147–69 doi:10.1002/elps.201300315

(https://www.ncbi.nlm.nih.gov/pubmed/24114758).

Garcia-Ruiz C, Garcia MA, Garcia MC, Marina ML (2006). Development of a capillary electrophoresis method for the determination of soybean proteins in soybean-rice gluten-free dietary products. Electrophoresis. 27(2):452–60 doi:10.1002/elps.200500355

(https://www.ncbi.nlm.nih.gov/pubmed/16342320).

George JW, O'Neill SL (2001). Comparison of refractometer and Biuret methods for total protein measurement in body cavity fluids. Vet Clin Pathol. 30(1):16–8 doi:10.1111/j.1939-165X.2001.tb00250.x

(https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1939-165X.2001.tb00250.x).

Godshall MA (1983). Interference of plant polysaccharides and tannin in the Coomassie Blue G250 test for protein. J Food Sci. 48(4):1346–7 doi:10.1111/j.1365-2621.1983.tb09227.x

(https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2621.1983.tb09227.x).

Goldring JPD (2012). Protein quantification methods to determine protein concentration prior to electrophoresis. In: Kurien BT, Scofield RH, editors. Protein electrophoresis: methods and protocols. Totowa, NJ: Humana Press, 29–35

(https://doi.org/10.1007/978-1-61779-821-4_3).

Goldring JPD (2015). Spectrophotometric methods to determine protein concentration. In: Kurien BT, Scofield RH, editors. Western blotting: methods and protocols. New York, NY: Springer New York, 41–7

(https://doi.org/10.1007/978-1-4939-2694-7_7).

Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB et al. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids. 50(12):1685–95 doi:10.1007/s00726-018-2640-5

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6245118/pdf/726_2018_Article_2640.pdf).

Gornall AG, Bardawill CJ, David MM (1949). Determination of serum proteins by means of the Biuret reaction. J Biol Chem. 177(2):751–66

(http://www.jbc.org/content/177/2/751.short).

Gouveia CSS, Freitas G, de Brito JH, Slaski JJ, de Carvalho MAAP (2014). Nutritional and mineral variability in 52 accessions of common bean varieties (Phaseolus vulgaris L.) from Madeira Island. J Agric Sci. 5:317–29

(https://dx.doi.org/10.4236/as.2014.54034).

Grappin R (1992). Bases and experiences of expressing the protein content of milk – France. J Dairy Sci. 75(11):3221–7

(https://www.sciencedirect.com/science/article/pii/S0022030292780886).

Grappin R, Jeunet R, Pillet R, Le Toquin A (1981). Etude des laits de chèvre. I. Teneur du lait de chèvre en matière grasse, matière azotée et fractions azotées. Lait. 61(603–604):117–33

(https://doi.org/10.1051/lait:1981603-6047).

Grassi S, Alamprese C (2018). Advances in NIR spectroscopy applied to process analytical technology in food industries. Curr Opinion Food Sci. 22:17–21

(https://www.sciencedirect.com/science/article/pii/S2214799317301534).

Hambraeus L, Forsum E, Abrahamsson L, Lonnerdal B (1976). Automatic total nitrogen analysis in nutritional evluations using a block digestor. Anal Biochem. 76(1–2):78–85 doi:10.1016/0003-2697(76)90508-X

(https://www.sciencedirect.com/science/article/pii/000326977690508X?via%3Dihub).

Hammersten O (1883). Zur frage ob caseín ein einheitlicher stoff sei. Zeitschrift für Physiologische Chemie 7.(7):227–73.

Hanko VP, Rohrer JS (2002). Direct determination of tryptophan using high-performance anionexchange chromatography with integrated pulsed amperometric detection. Anal Biochem. 30:204–9

(https://www.ncbi.nlm.nih.gov/pubmed/12419331).

Hayashi R, Suzuki F (1985). Determination of methionine sulfoxide in protein and food by hydrolysis with p-toluenesulfonic acid. Anal Biochem. 149:521–8

(https://www.sciencedirect.com/science/article/pii/0003269785906086?via%3Dihub).

Heathcote JG (1950). The protein quality of oats. Br J Nutr. 4(2-3):145–54

(https://www.ncbi.nlm.nih.gov/pubmed/14801413).

Heidelbaugh ND, Huber CS, Bednarczyk JF, Smith MC, Rambaut PC, Wheeler HO (1975). Comparison of three methods for calculating protein content of foods. J Agric Food Chem. 23(4):611–3 doi:10.1021/jf60200a006

(https://pubs.acs.org/doi/pdfplus/10.1021/jf60200a006).

Hejtmankova A, Pivec V, Trnkova E, Dragounova H (2012). Differences in the composition of total and whey proteins in goat and ewe milk and their changes throughout the lactation period. Czech J Anim Sci. 57(7):323–31 doi:10.17221/6007-cjas

(https://www.agriculturejournals.cz/publicFiles/68159.pdf).

Hemstrom P, Irgum K (2006). Hydrophilic interaction chromatography. J Sep Sci. 29(12):1784–821

(https://onlinelibrary.wiley.com/doi/abs/10.1002/jssc.200600199).

Hendriks WH, Moughan PJ, Boer H, van der Poel AFB (1994). Effects of extrusion on the dyebinding, fluorodinitrobenzene-reactive and total lysine content of soyabean meal and peas. Anim Feed Sci Technol. 48:99–109

(https://www.sciencedirect.com/science/article/pii/0377840194901147).

Hiroyuki N, Terabe S (1996). Micellar electrokinetic chromatography perspectives in drug analysis. J Chromatogr A. 735(1):3–27

(https://www.sciencedirect.com/science/article/pii/0021967395013814).

Holt NW, Sosulski FW (1979). Amino acid composition and protein quality of field peas. Can J Plant Sci. 59(3):653–60

(https://search.ebscohost.com/login.aspx?direct=true&db=ffh&AN=1980-05-J-0633&lang=fr&site=ehost-live).

Horneck DA, Miller RO (1998). Determination of total nitrogen in plant tissue. In: Kalra YP, editor. Handbook of reference methods for plant analysis. Boca Raton: CRC Press, 75–83

(https://www.scirp.org/(S(vtj3fa45qm1ean45vvffcz55))/reference/ReferencesPapers.aspx?ReferenceID=2116158).

Hughes GJ, Ryan DJ, Mukherjea R, Schasteen CS (2011). Protein digestibility-corrected amino acid scores (PDCAAS) for soy protein isolates and concentrate: criteria for evaluation. J Agric Food Chem. 59(23):12707–12 doi:10.1021/jf203220v

(https://pubs.acs.org/doi/10.1021/jf203220v).

Hurrell RF, Carpenter KJ (1974). Mechanisms of heat damage in proteins. 4. The reactive lysine content of heat-damaged material as measured in different ways. Br J Nutr. 32:589–604

(https://www.ncbi.nlm.nih.gov/pubmed/4433504).

IDF (2016). Evaluation of nitrogen conversion factors for dairy and soy. Bull Int Dairy Fed. (482):1–38

(https://store.fil-idf.org/product/bulletin-idf-n-482-2016-evaluation-nitrogen-conversion-factors-dairy-soy/).

Jager AV, Tonin FG, Tavares MFM (2007). Comparative evaluation of extraction procedures and method validation for determination of carbohydrates in cereals and dairy products by capillary electrophoresis. J Sep Sci. 30(4):586–94 doi:10.1002/jssc.200600370

(https://onlinelibrary.wiley.com/doi/abs/10.1002/jssc.200600370).

Jonas-Levi A, Martinez JJI (2017). The high level of protein content reported in insects for food and feed is overestimated. J Food Compos Anal. 62:184–8 doi:10.1016/j.jfca.2017.06.004

(https://www.sciencedirect.com/science/article/pii/S0889157517301424?via%3Dihub).

Jones D (1931). Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. US Dept Agric, Circular Series. 183:1–21

(http://foodfacts.foodcomp.info/References/Protein/Jones_1941%20nitrogen-protein%20conversion%20cir183.pdf).

Journet M, Vérité R, Vignon B (1975). L'azote non protéique du lait : facteurs de variation. Lait. 55(543–544):212–23

(https://doi.org/10.1051/lait:1975543-54414).

Kaisoon O, Siriamornpun S, Meeso N (2008). Distinction between cereal genotypes based on the protein and DNA composition of the grain by capillary electrophoresis. World Appl Sci J. 4(3):384–95

(https://pdfs.semanticscholar.org/a939/b90006daef7ab834f64ba6a7ffdcf793e1c5.pdf).

Kamath P, Pattabiraman TN (1988). Phenols interfere in protein estimation by the bicinchoninic acid assay-method. Biochem Arch. 4(1):17–23

(https://eurekamag.com/research/006/101/006101130.php).

Karman AH, van Boekel MAJS (1986). Evaluation of the Kjeldahl factor for conversion of the nitrogen content of milk and milk products to protein content. Neth Milk Dairy J. 40:315–36.

Katsoulos PD, Athanasiou LV, Karatzia MA, Giadinis N, Karatzias H, Boscos C et al. (2017). Comparison of Biuret and refractometry methods for the serum total proteins measurement in ruminants. Vet Clin Pathol. 46(4):620–4 doi:10.1111/vcp.12532

(https://onlinelibrary.wiley.com/doi/abs/10.1111/vcp.12532).

Kaushal V, Barnes LD (1986). Effect of zwitterionic buffers on measurement of small masses of protein with bicinchoninic acid. Anal Biochem. 157(2):291–4

(https://www.sciencedirect.com/science/article/pii/0003269786906299).

Keller RP, Neville MC (1986). Determination of total protein in human milk: comparison of methods. Clin Chem. 32(1):120–3

(http://clinchem.aaccjnls.org/content/clinchem/32/1/120.full.pdf).

Kenndler E (2014a). A critical overview of non-aqueous capillary electrophoresis. Part I: mobility and separation selectivity. J Chromatogr A. 1335:16–30

(https://www.sciencedirect.com/science/article/pii/S0021967314000521).

Kenndler E (2014b). A critical overview of non-aqueous capillary electrophoresis. Part II: separation efficiency and analysis time. J Chromatogr A. 1335:31–41

(https://www.sciencedirect.com/science/article/pii/S0021967314000922).

Kessler RJ, Fanestil DD (1986). Interference by lipids in the determination of protein using bicinchoninic acid. Anal Biochem. 159(1):138–42

(https://www.sciencedirect.com/science/article/pii/0003269786903180).

Khan MY, Newman SA (1990). An assay for heparin by decrease in color yield (DECOY) of a protein-dye-binding reaction. Anal Biochem. 187(1):124–8

(https://www.sciencedirect.com/science/article/pii/000326979090428C).

Kitagishi K, Shintani H (1998). Analysis of compounds containing carboxyl groups in biological fluids by capillary electrophoresis. J Chromatogr B: Biomed Sci App. 717(1):327–39

(https://www.sciencedirect.com/science/article/pii/S0378434798002904).

Kjeldahl J (1883). New method for the determination of nitrogen. Chem News. 48(1240):101–2.

Klosterman LJ (1985). A research school of chemistry in the nineteenth century: Jean Baptiste Dumas and his research students. Ann Sci. 42(1):41–80 doi:10.1080/00033798500200111

(https://doi.org/10.1080/00033798500200111).

Krizova L, Hanus O, Roubal P, Kucera J, Hadrova S (2013). The effect of cattle breed, season and type of diet on nitrogen fractions and amino acid profile of raw milk. Archiv Tierzucht. 56:709–18 doi:10.7482/0003-9438-56-071

(https://www.arch-anim-breed.net/56/709/2013/aab-56-709-2013.pdf).

Krul ES (2019). Calculation of nitrogen-to-protein conversion factors: a review with a focus on soy protein. J Am Oil Chem Soc. 96(4):339–64 doi:10.1002/aocs.12196

(https://aocs.onlinelibrary.wiley.com/doi/abs/10.1002/aocs.12196).

Kwanyuen P, Burton JW (2010). A modified amino acid analysis using PITC derivatization for soybeans with accurate determination of cysteine and half-cystine. J Am Oil Chem Soc. 87(2):127–32 doi:10.1007/s11746-009-1484-2

(https://aocs.onlinelibrary.wiley.com/doi/abs/10.1007/s11746-009-1484-2).

La Barbera G, Capriotti AL, Cavaliere C, Montone CM, Piovesana S, Samperi R et al. (2017). Liquid chromatography-high resolution mass spectrometry for the analysis of phytochemicals in vegetal-derived food and beverages. Food Res Int. 100:28–52

(https://www.sciencedirect.com/science/article/pii/S0963996917304143).

Lakin AL (1978). Determination of nitrogen and estimation of protein in foods. In: King RD, editor. Developments in Food Analysis Techniques. London: Applied Science Publishers, 43–74.

Landry J, Delhaye S, Jones DG (1992). Determination of tryptophan in feedstuffs: comparison of two methods of hydrolysis prior to HPLC analysis. J Sci Food Agric. 58:439–41

(https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.2740580321).

Lara FJ, Garcia-Campana AN, Alés-Barrero F, Bosque-Sendra JM, Garcia-Ayuso LE (2006). Multiresidue method for the determination of quinolone antibiotics in bovine raw milk by capillary electrophoresis-tandem mass spectrometry. Anal Chem. 78:7665–73 doi:10.1021/ac061006v

(https://pubs.acs.org/doi/pdfplus/10.1021/ac061006v).

Laurens LML, Olstad JL, Templeton DW (2018). Total protein content determination of microalgal biomass by elemental nitrogen analysis and a dedicated nitrogen-to-protein conversion factor. Method Mol Biol. doi:10.1007/7651_2018_126

(https://link.springer.com/protocol/10.1007%2F7651_2018_126).

Leca-Bouvier B, Blum LJ (2005). Biosensors for protein detection: a review. Anal Lett. 38(10):1491–517 doi:10.1081/al-200065780

(https://www.tandfonline.com/doi/abs/10.1081/AL-200065780).

Lee KS, Drescher DG (1978). Fluorometric amino-acid analysis with o-phthaldialdehyde (OPA). Int J Biochem. 9(7):457–67

(https://www.sciencedirect.com/science/article/pii/0020711X78900757).

Legler G, Muller-Platz CM, Mentges-Hettkamp M, Pflieger G, Julicj E (1985). On the chemical basis of the Lowry protein determination. Anal Biochem. 150(2):278–87

(https://www.sciencedirect.com/science/article/pii/0003269785905111?via%3Dihub).

Linker R, Etzion Y (2009). Potential and limitation of mid-infrared attenuated total reflectance spectroscopy for real time analysis of raw milk in milking lines. J Dairy Res. 76(1):42–8 doi:10.1017/S0022029908003580

(https://www.ncbi.nlm.nih.gov/pubmed/18925993).

Liu JC, Feng H, He JQ, Chen HX, Ding DY (2018). The effects of nitrogen and water stresses on the nitrogen-to-protein conversion factor of winter wheat. Agric Water Manag. 210:217–23 doi:10.1016/j.agwat.2018.07.042

(https://www.sciencedirect.com/science/article/pii/S0378377418311375?via%3Dihub).

Lleu PL, Rebel G (1991). Interference of Good's buffers and other biological buffers with protein determination. Anal Biochem. 192(1):215–8

(https://www.sciencedirect.com/science/article/pii/000326979190210K).

López-Lorente ÁI, Mizaikoff B (2016). Mid-infrared spectroscopy for protein analysis: potential and challenges. Anal Bioanal Chem. 408(11):2875–89 doi:10.1007/s00216-016-9375-5

(https://doi.org/10.1007/s00216-016-9375-5).

Lott JA, Stephan VA, Pritchard KAJ (1983). Evaluation of the Coomassie Brilliant Blue G-250 method for urinary protein. Clin Chem. 29(11):1946–50

(http://clinchem.aaccjnls.org/content/29/11/1946.abstract).

Lourenço SO, Barbarino E, De-Paula JC, da S. Pereira OL, Lanfer Marquez UM (2002). Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycol Res. 50:233–41

(https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1440-1835.2002.00278.x).

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J Biol Chem. 193:265–75

(http://www.jbc.org/content/193/1/265.full.pdf).

Lu BC, Ting (2004). Algorithms for de novo peptide sequencing using tandem mass spectrometry. Drug Discovery Today: Biosilico. 2(2): 85–90 doi:10.1016/S1741-8364(04)02387-X.

Lu W, Lv X, Gao B, Shi H, Yu LL (2015). Differentiating milk and non-milk proteins by UPLC amino acid fingerprints combined with chemometric data analysis techniques. J Agric Food Chem. 63(15):3996–4002 doi:10.1021/acs.jafc.5b00702

(https://pubs.acs.org/doi/10.1021/acs.jafc.5b00702).

Luginbühl W (2002). Evaluation of designed calibration samples for casein calibration in fourier transform infrared analysis of milk. LWT – Food Sci Tech. 35(6):554–8

(https://www.sciencedirect.com/science/article/pii/S0023643802909023).

Lynch JM, Barbano DM (1999). Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. J AOAC Int. 82(6):1389–98

(https://www.ncbi.nlm.nih.gov/pubmed/10589493).

Lynch JM, Barbano DM, Fleming JR (1998). Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen analysis: collaborative study. J AOAC Int. 81(4):763–74

(https://www.ncbi.nlm.nih.gov/pubmed/9680702).

Mala Z, Gebauer P, Bocek P (2015). Recent progress in analytical capillary isotachophoresis. Electrophoresis. 36(1):2–14 doi:10.1002/elps.201400337

(https://www.ncbi.nlm.nih.gov/pubmed/25130397).

Mala Z, Gebauer P, Bocek P (2016). Analytical capillary isotachophoresis after 50 years of development: recent progress 2014–2016. Electrophoresis. 38(1):9–19 doi:10.1002/elps.201600289

(https://www.ncbi.nlm.nih.gov/pubmed/27449988).

Mao L-C, Lee K-H, Erbersbobler HF (1993). Effects of heat treatment on lysine in soya protein. J Sci Food Agric. 62:307–9

(https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.2740620316).

Marino R, Iammarino M, Santillo A, Muscarella M, Caroprese M, Albenzio M (2010). Technical note: rapid method for determination of amino acids in milk. J Dairy Sci. 93(6):2367–70 doi:10.3168/jds.2009-3017

(https://www.ncbi.nlm.nih.gov/pubmed/20494144).

Mariotti F, Tome D, Mirand PP (2008). Converting nitrogen into protein – beyond 6.25 and Jones' factors. Crit Rev Food Sci Nutr. 48(2):177–84 doi:10.1080/10408390701279749

(https://www.tandfonline.com/doi/pdf/10.1080/10408390701279749?needAccess=true).

Marshall T, Williams KM (1991). Drug interference in the Bradford and 2,2´-bicinchoninic acid protein assays. Anal Biochem. 198(2):352–4

(https://www.sciencedirect.com/science/article/pii/000326979190438Y).

Martens DA, Reedy TE, Lewis DT (2004). Soil organic carbon content and composition of 130-year crop, pasture and forest land-use managements. Global Change Biol. 10(1):65–78 doi:10.1046/j.1529-8817.2003.00722.x

(https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1529-8817.2003.00722.x).

Martin-Girardeau A, Renou-Gonnord M-F (2000). Optimization of a capillary electrophoresis-electrospray mass spectrometry method for the quantitation of the 20 natural amino acids in childrens blood. J Chromatogr B: Biomed Sci App. 742(1):163–71

(https://www.sciencedirect.com/science/article/pii/S0378434700001572).

Martina V, Vojtech K (2015). A comparison of Biuret, Lowry and Bradford methods for measuring the egg's proteins.

(https://mnet.mendelu.cz/mendelnet2015/articles/62_vrsanska_1167.pdf).

Mattila P, Salo-Vaananen P, Konko K, Aro H, Jalava T (2002). Basic composition and amino acid contents of mushrooms cultivated in Finland. J Agric Food Chem. 50(22):6419–22

(https://pubs.acs.org/doi/pdfplus/10.1021/jf020608m).

Maubois JL, Lorient D (2016). Dairy proteins and soy proteins in infant foods nitrogen-to-protein conversion factors. Dairy Sci Technol. 96:15–25 doi:10.1007/s13594-015-0271-0

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4701760/pdf/13594_2015_Article_271.pdf).

Mauron J, Bujard E (1964). Guanidination, an alternative approach to the determination of available lysine in foods. Edinburgh, UK, Proc Natl Acad Sci.

McDermott A, De Marchi M, Berry DP, Visentin G, Fenelon MA, Lopez-Villalobos N et al. (2017). Cow and environmental factors associated with protein fractions and free amino acids predicted using mid-infrared spectroscopy in bovine milk. J Dairy Sci. 100(8):6272–84 doi:10.3168/jds.2016-12410

(https://www.sciencedirect.com/science/article/pii/S0022030217305921?via%3Dihub).

Meng L, Shen G, Hou X, Wang L (2009). Determination of melamine in food by SPE and CZE with UV detection. Chromatographia. 70(5):991–4 doi:10.1365/s10337-009-1249-y

(https://doi.org/10.1365/s10337-009-1249-y).

Merrick BA, London RE, Bushel PR, Grissom SF, Paules RS (2011). Platforms for biomarker analysis using high-throughput approaches in genomics, transcriptomics, proteomics, metabolomics, and bioinformatics. IARC Sci Publ.(163):121–42

(https://www.ncbi.nlm.nih.gov/pubmed/22997859).

Mihaljev ŽA, Jakšić SM, Prica NB, Ćupić ŽN, Živkov-Baloš MM (2015). Comparison of the Kjeldahl method, Dumas method and NIR method for total nitrogen determination in meat and meat products. J Agroaliment Processes Technol. 21(4):365–70

(https://www.journal-of-agroalimentary.ro/Journal-of-Agroalimentary-Processes-and-Technologies-Article_77515y.html).

Mikšík I (2018). Capillary gel and sieving electrophoresis. In: Poole CF, editor. Capillary electromigration separation methods. Amsterdam: Elsevier, 143–65

(https://www.sciencedirect.com/science/article/pii/B978012809375700006X).

Mohammad FK, Stomer VEV (1991). Measurement of total plasma-protein concentration in lambs, cows and dogs by the Bradford dye-binding method and a comparison with the standard Biuret method. Indian J Anim Sci. 61(6):615–6.

Molnár-Perl I (2003). Quantitation of amino acids and amines in the same matrix by high-performance liquid chromatography, either simultaneously or separately. J Chromatogr A. 987(1):291–309

(https://www.sciencedirect.com/science/article/pii/S0021967302015376).

Moore JC, DeVries JW, Lipp M, Griffiths JC, Abernethy DR (2010). Total protein methods and their potential utility to reduce the risk of food protein adulteration. CRFSFS. 9(4):330–57 doi:10.1111/j.1541-4337.2010.00114.x

(https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1541-4337.2010.00114.x).

Moore S, Stein WH (1948). Photometric ninhydrin method for use in the chromatography of amino acids. J Biol Chem. 176(1):367–88

(https://www.ncbi.nlm.nih.gov/pubmed/18886175).

Moore S, Stein WH (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem. 211(2):907–13

(http://www.jbc.org/content/211/2/907.full.pdf).

Morr CV (1981). Nitrogen conversion factors for several soybean protein products. J Food Sci. 46(5):1362 doi:10.1111/j.1365-2621.1981.tb04175.x

(https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2621.1981.tb04175.x).

Morr CV (1982). Recalculated nitrogen conversion factors for several soybean protein products. J Food Sci. 47(5):1751–2 doi:10.1111/j.1365-2621.1982.tb05032.x

(https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2621.1982.tb05032.x).

Morries P (1983). A century of Kjeldahl (1883–1893). J Assoc Public Anal. 19:493–505.

Mossé J (1990). Nitrogen to protein conversion factor for ten cereals [wheat, triticale, barley, maize, sorghum, rice, pearl millet, oats, rye, Setaria italica] and six legumes or oilseeds [peas, lupins, soyabeans, Vicia faba, sunflowers, Phaseolus vulgaris]. A reappraisal of its definition and determination. Variation according to species and seed protein content. J Agric Food Chem. 38(1):18–24 doi:10.1021/jf00091a004

(https://pubs.acs.org/doi/pdfplus/10.1021/jf00091a004).

Mossé J, Huet JC, Baudet J (1985). The amino acid composition of wheat grain as a function of nitrogen content. J Cereal Sci. 3(2):115–30

(https://www.sciencedirect.com/science/article/pii/S0733521085800229).

Mossé J, Huet JC, Baudet J (1988). The amino acid composition of rice grain as a function of nitrogen content as compared with other cereals: a reappraisal of rice chemical scores. J Cereal Sci. 8(2):165–75

(https://www.sciencedirect.com/science/article/pii/S0733521088800274).

Müller J (2017). Dumas or Kjeldahl for reference analysis? Comparison and considerations for nitrogen/protein analysis of food and feed. FOSS Analytics. White paper

(https://www.fossanalytics.com/-/media/files/documents/papers/laboratories-segment/the-dumas-method-for-nitrogenprotein-analysis_gb.pdf).

Nakai S, Le AC (1970). Spectrophotometric determination of protein and fat in milk simultaneously. J Dairy Sci. 53(3):276–8

(https://www.sciencedirect.com/science/article/pii/S0022030270861975).

Nazzaro F, Orlando P, Fratianni F, Di Luccia A, Coppola R (2012). Protein analysis-on-chip systems in foodomics. Nutrients. 4(10):1475–89

(https://res.mdpi.com/nutrients/nutrients-04-01475/article_deploy/nutrients-04-01475.pdf?filename=&attachment=1).

Niall H (1973). Automated Edman degradation: the protein sequenator. Method Enzymol. 27:942–1010 doi:10.1016/S0076-6879(73)27039-8

(https://www.sciencedirect.com/science/article/pii/S0076687973270398).

Nissen S (1992). Amino acid analysis in food and physiological samples. In: Nissen S, editor. Modern methods in protein nutrition and metabolism. San Diego: Academic Press, 1–8.

Noble JE, Bailey MJA (2009). Quantitation of protein. Method Enzymol. 463:73–95

(https://www.sciencedirect.com/science/article/pii/S0076687909630081).

Nouadje G, Rubie H, Chatelut E, Canal P, Nertz M, Puig P et al. (1995). Child cerebrospinal fluid analysis by capillary electrophoresis and laser-induced fluorescence detection. J Chromatogr A. 717(1):293–8

(https://www.sciencedirect.com/science/article/pii/0021967395007473).

O'Riordan N, Kane M, Joshi L, Hickey RM (2014). Structural and functional characteristics of bovine milk protein glycosylation. Glycobiol. 24(3):220–36 doi:10.1093/glycob/cwt162

(https://doi.org/10.1093/glycob/cwt162).

O'Donnell R, Holland JW, Deeth HC, Alewood P (2004). Milk proteomics. Int Dairy J. 14(12):1013–23

(https://www.sciencedirect.com/science/article/pii/S0958694604000871).

Okutucu B, Dınçer A, Habib Ö, Zıhnıoglu F (2007). Comparison of five methods for determination of total plasma protein concentration. J Biochem Bioph Meth. 70(5):709–11

(https://www.sciencedirect.com/science/article/pii/S0165022X07001145).

Otter DE (2012). Standardised methods for amino acid analysis of food. Br J Nutr. 108(S2):S230–S7 doi:10.1017/S0007114512002486

(https://www.ncbi.nlm.nih.gov/pubmed/23107533).

Owusu-Apenten RK (2002). Food protein analysis: quantitative effects on processing. New York: Marcel Dekker

(http://154.68.126.6/library/Food%20Science%20books/batch1/Food%20protein%20analysis%20-%20quantitative%20effects%20on%20processing.pdf).

Ozols J (1990). Amino acid analysis. Method Enzymol. 182:587–601

(https://www.sciencedirect.com/science/article/pii/0076687990820465).

Pande SV, Murthy MSR (1994). A modified micro-Bradford procedure for elimination of interference from sodium dodecyl sulfate, other detergents, and lipids. Anal Biochem. 220(2):424–6

(https://www.sciencedirect.com/science/article/pii/S0003269784713613).

Pasquini C (2018). Near infrared spectroscopy: a mature analytical technique with new perspectives – a review. Anal Chim Acta. 1026:8–36

(https://www.sciencedirect.com/science/article/pii/S0003267018304793).

Peace RW, Gilani GS (2005). Chromatographic determination of amino acids in foods. J AOAC Int. 88(3):877–87

(https://www.ingentaconnect.com/content/aoac/jaoac/2005/00000088/00000003/art00029).

Peterson GL (1979). Review of the folin phenol protein quantitation of Lowry, Rosebrough, Farr and Randall. Anal Biochem. 100(2):201–20

(https://www.sciencedirect.com/science/article/pii/0003269779902227?via%3Dihub).

Petterson DS, Harris DJ, Rayner CJ, Blakeney AB, Choct M (1999). Methods for the analysis of premium livestock grains. Aust J Agr Res. 50(5):775–87

(https://hdl.handle.net/2123/2264).

Pinero MY, Bauza R, Arce L (2011). Thirty years of capillary electrophoresis in food analysis laboratories: potential applications. Electrophoresis. 32(11):1379–93 doi:10.1002/elps.201000541

(https://www.ncbi.nlm.nih.gov/pubmed/21538397).

Porep JU, Kammerer DR, Carle R (2015). On-line application of near infrared (NIR) spectroscopy in food production. Trends Food Sci Tech. 46(2A):211–30

(https://www.sciencedirect.com/science/article/pii/S0924224415002174).

Przyborowska AM, Waterman D, Halket JM, Fraser PD, Bramley PM, Patel RKP (2004). Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. J Exp Bot. 56(410):219–43 doi:10.1093/jxb/eri069

(https://doi.org/10.1093/jxb/eri069).

Rafecas I, Esteve M, Fernandezlopez JA, Remesar X, Alemany M (1994). Whole-rat protein-content estimation – applicability of the N-x-6.25 factor. Br J Nutr. 72(2):199–209 doi:10.1079/bjn19940024

(https://www.ncbi.nlm.nih.gov/pubmed/7947640).

Rao SR, Carter FL, Frampton VL (1963). Determination of available lysine in oilseed meal proteins. Anal Chem. 35:1927–30

(https://pubs.acs.org/doi/abs/10.1021/ac60205a042).

Rayner CJ (1985). Protein hydrolysis of animal feeds for amino acid content. J Agric Food Chem. 33(4):722–5 doi:10.1021/jf00064a039

(https://pubs.acs.org/doi/pdfplus/10.1021/jf00064a039).

Redmile-Gordon MA, Armenise E, White RP, Hirsch PR, Goulding KWT (2013). A comparison of two colorimetric assays, based upon Lowry and Bradford techniques, to estimate total protein in soil extracts. Soil Biol Biochem. 67:166–73

(https://www.sciencedirect.com/science/article/pii/S0038071713002824).

Ribadeau-Dumas B, Grappin R (1989). Milk protein analysis. Lait (Lyon). 69(5):357–416

(https://hal.archives-ouvertes.fr/hal-00929173/document).

Righetti PG (2005). Electrophoresis: the march of pennies, the march of dimes. J Chromatogr A. 1079(1):24–40

(https://www.sciencedirect.com/science/article/pii/S0021967305000877).

Robinson T (1979). The determination of proteins in plant extracts that contain polyphenols. Plant Sci Letters. 15(3):211–6

(https://www.sciencedirect.com/science/article/pii/0304421179901123).

Rodriguez-Otero JL, Hermida M, Cepeda A (1995). Determination of fat, protein, and total solids in cheese by near-infrared reflectance spectroscopy. J AOAC Int. 78(3):802–6

(https://www.researchgate.net/publication/15442086_Determination_of_fat_protein_and_total_solids_in_cheese_by_near-infrared_reflectance_spectroscopy).

Ronalds JA, O'Brien L, Allen SJ (1984). Determination of the proportion of acetic acid-insoluble protein in wheat flours by alkaline steam distillation. J Cereal Sci. 2(1):25–9

(https://www.sciencedirect.com/science/article/pii/S0733521084800041).

Rouch DA, Roginski H, Britz ML, Roupas P (2008). Determination of a nitrogen conversion factor for protein content in cheddar cheese. Int Dairy J. 18(2):216–20 doi:10.1016/j.idairyj.2007.07.004

(https://www.sciencedirect.com/science/article/pii/S0958694607001343?via%3Dihub).

Rouch DA, Roupas P, Roginski H (2007). True protein value of milk and dairy products. Aust J Dairy Tech. 62(1):26–30.

Rowan AM, Moughan PJ, Wilson MN (1989). Alkaline hydrolysis for the determination of tryptophan in biological samples. Proc Nutr Soc. 14:169–72.

Rutherfurd SM, Gilani GS (2009). Amino acid analysis. Curr Protoc Protein Sci. 58(1):11.9.1–37 doi:10.1002/0471140864.ps1109s58

(https://currentprotocols.onlinelibrary.wiley.com/doi/abs/10.1002/0471140864.ps1109s58).

Rutherfurd SM, Moughan PJ (1997). Determining reactive lysine and the digestibility of reactive lysine in heat processed foods. In: Savage GP, editor. Proc Nutr Soc. Canterbury: Nutrition Society of New Zealand, 222–34.

Rutherfurd SM, Moughan PJ (1998). The digestible amino acid composition of several milk proteins: application of a new bioassay. J Dairy Sci. 81(4):909–17 doi:10.3168/jds.S0022-0302(98)75650-4

(https://www.sciencedirect.com/science/article/pii/S0022030298756504?via%3Dihub).

Rutherfurd SM, Moughan PJ (2018). The chemical analysis of proteins and amino acids. In: Moughan PJ, Hendriks WH, editors. Feed Evaluation Science. Wageningen: Wageningen Academic Publishers.

Sáez-Plaza P, Michałowski T, Navas M, Asuero GA, Wybraniec S (2013a). An overview of the Kjeldahl method of nitrogen determination. Part I. Early history, chemistry of the procedure, and titrimetric finish. Crit Rev Anal Chem. 43(4):178–223 doi:10.1080/10408347.2012.751786

(https://www.tandfonline.com/doi/abs/10.1080/10408347.2012.751786).

Sáez-Plaza P, Navas M, Wybraniec S, Michałowski T, Asuero GA (2013b). An overview of the Kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish, and quality control. Crit Rev Anal Chem. 43(4):224–72 doi:10.1080/10408347.2012.751787

(https://www.tandfonline.com/doi/abs/10.1080/10408347.2012.751787).

Saint-Denis T, Goupy J (2004). Optimization of a nitrogen analyser based on the Dumas method. Anal Chim Acta. 515(1):191–8

(https://www.sciencedirect.com/science/article/pii/S0003267003014673).

Salo-väänänen PP, Koivistoinen PE (1996). Determination of protein in foods: comparison of net protein and crude protein (N × 6.25) values. Food Chem. 57(1):27–31

(https://www.sciencedirect.com/science/article/pii/0308814696001574).

Samejima K, Dairman W, Stone J, Udenfriend S (1971). Condensation of ninhydrin with aldehydes and primary amines to yield highly fluorescent ternary products. II. Application to the detection and assay of peptides, amino acids, amines, and amino sugars. Anal Biochem. 42(1):237–47

(https://www.sciencedirect.com/science/article/pii/0003269771900315?via%3Dihub).

Sánchez-Rivera L, Martínez-Maqueda D, Cruz-Huerta E, Miralles B, Recio I (2014). Peptidomics for discovery, bioavailability and monitoring of dairy bioactive peptides. Food Res Int. 63:170–81

(https://www.sciencedirect.com/science/article/pii/S0963996914000982).

Santos B, Lista A, Simonet BM, Rios A, Valcarcel M (2005). Screening and analytical confirmation of sulfonamide residues in milk by capillary electrophoresis-mass spectrometry. Electrophoresis. 26(7–8):1567–75 doi:10.1002/elps.200410267

(https://www.ncbi.nlm.nih.gov/pubmed/15765476).

Sapan CV, Lundblad RL (2015). Review of methods for determination of total protein and peptide concentration in biological samples. Proteomics Clin Appl. 9(3–4):268–76 doi:10.1002/prca.201400088

(https://onlinelibrary.wiley.com/doi/abs/10.1002/prca.201400088).

Sarwar G, Blair R, Friedman M, Gumbmann MR, Hackler LR, Pellett PL et al. (1983a). Comparison of interlaboratory variation in amino acid analysis and rat growth assays for evaluating protein quality. J Assoc Off Anal Chem. 68(1):52–6.

Sarwar G, Christensen DA, Finlayson AJ, Friedman M, Hackler LR, Mackenzie SL et al. (1983b). Inter- and intra-laboratory variation in amino acid analysis of food proteins. J Food Sci. 48(2):526–31 doi:10.1111/j.1365-2621.1983.tb10781.x

(https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2621.1983.tb10781.x).

Sarwar G, Sosulski FW, Bell JM (1973). Nutritional evaluation of oilseed meals and protein isolates by mice. Can Inst Food Sci Technol J. 6(1):17–21

(https://www.sciencedirect.com/science/article/pii/S0315546373739584).

Searle PL (1984). The berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. Analyst. 109(5):549–68.

Simonian MH (2002). Spectrophotometric determination of protein concentration. Curr Protoc Cell Bio. 15(1):A.3B.1–7 doi:10.1002/0471143030.cba03bs15

(https://currentprotocols.onlinelibrary.wiley.com/doi/abs/10.1002/0471143030.cba03bs15).

Simonian MH, Smith JA (2006). Spectrophotometric and colorimetric determination of protein concentration. Curr Protoc Mol Biol. 76(1):10.1.1 doi:10.1002/0471142727.mb1001as76

(https://currentprotocols.onlinelibrary.wiley.com/doi/abs/10.1002/0471142727.mb1001as76).

Simonne AH, Simonne EH, Eitenmiller RR, Mills HA, Cresman CP, III (1997). Could the Dumas method replace the Kjeldahl digestion for nitrogen and crude protein determinations in foods? JSci Food Ag. 73(1):39–45 doi:10.1002/(SICI)1097-0010(199701)73:1<39::AID-JSFA717>3.0.CO;2-4

(https://onlinelibrary.wiley.com/doi/abs/10.1002/%28SICI%291097-0010%28199701%2973%3A1%3C39%3A%3AAID-JSFA717%3E3.0.CO%3B2-4).

Simonne EH, Mills HA, Jones JB, Smittle DA, Hussey CG (1994). A comparison of analytical methods for nitrogen analysis in plant tissues. Commun Soil Sci Plant Anal. 25(7–8):943–54 doi:10.1080/00103629409369090

(https://doi.org/10.1080/00103629409369090).

Slocum TL, Deupree JD (1991). Interference of biogenic amines with the measurement of proteins using bicinchoninic acid. Anal Biochem. 195(1):14–7

(https://www.sciencedirect.com/science/article/pii/0003269791902874).

Smart KF, Aggio RBM, Van Houtte JR, Villas-Bôas SG (2010). Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography–mass spectrometry. Nat Protoc. 5:1709 doi:10.1038/nprot.2010.108

(https://doi.org/10.1038/nprot.2010.108).

Soby LM, Johnson P (1981). Determination of asparagine and glutamine in polypeptides using bis(1,1-trifluoroacetoxy)iodobenzene. Anal Biochem. 113:149–53

(https://www.sciencedirect.com/science/article/pii/0003269781900580?via%3Dihub).

Sochaski MA, Jenkins AJ, Lyons TJ, Thorpe SR, Baynes JW (2001). Isotope dilution gas chromatography/mass spectrometry method for the determination of methionine sulfoxide in protein. Anal Chem. 73:4662–7

(https://www.ncbi.nlm.nih.gov/pubmed/11605844).

Soga T, Heiger DN (2000). Amino acid analysis by capillary electrophoresis electrospray ionization mass spectrometry. Anal Chem. 72(6):1236–41.

Sorensen LK, Lund M, Juul B (2003). Accuracy of Fourier transform infrared spectrometry in determination of casein in dairy cows' milk. J Dairy Res. 70(4):445–52

(https://www.ncbi.nlm.nih.gov/pubmed/14649416).

Sosulski FW, Holt NW (1980). Amino acid composition and nitrogen-to-protein factors for grain legumes. Can J Plant Sci. 60(4):1327–31

(https://search.ebscohost.com/login.aspx?direct=true&db=ffh&AN=1982-03-J-0356&lang=fr&site=ehost-live).

Sosulski FW, Imafidon GI (1990). Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J Agric Food Chem. 38:1351–6.

Sosulski FW, Sarwar G (1973). Amino acid composition of oilseed meals and protein isolates. Can Inst Food Sci Technol J. 6(1):1–5

(https://search.ebscohost.com/login.aspx?direct=true&db=ffh&AN=1974-03-G-0117&lang=fr&site=ehost-live).

Sriperm N, Pesti GM, Tillman PB (2011). Evaluation of the fixed nitrogen-to-protein (N:P) conversion factor (6.25) versus ingredient specific N:P conversion factors in feedstuffs. J Sci Food Agric. 91(7):1182–6 doi:10.1002/jsfa.4292

(https://onlinelibrary.wiley.com/doi/pdf/10.1002/jsfa.4292).

Steinhart H (1984). Summary of the workshop on "tryptophan analysis". In: Zebrowska T, Buraczewska L, Buraczewski S, Kowalczyk J et al., editors Proceedings of the VI International Symposium on Amino Acids, Warsaw. Polish Scientific Publishers; 434–47.

Stodt U, Engelhardt UH (2013). Progress in the analysis of selected tea constituents over the past 20 years. Food Res Int. 53(2):636–48

(https://www.sciencedirect.com/science/article/pii/S0963996913000124).

Svec F (2009). CEC: selected developments that caught my eye since the year 2000. Electrophoresis. 30(S1):S68–82 doi:10.1002/elps.200900062

(https://www.ncbi.nlm.nih.gov/pubmed/19517503).

Szabadváry F, Robinson A (1980). The history of analytical chemistry. In: Svehla G, editor. Compr Anal Chem. Amsterdam: Elsevier, 61–282

(https://www.sciencedirect.com/science/article/pii/B9780444418593500086).

Templeton DW, Laurens LML (2015). Nitrogen-to-protein conversion factors revisited for applications of microalgal biomass conversion to food, feed and fuel. Algal Res. 11:359–67 doi:10.1016/j.algal.2015.07.013

(https://www.sciencedirect.com/science/article/pii/S2211926415300230).

Terabe S (2009). Capillary separation: micellar electrokinetic chromatography. Ann Rev Anal Chem. 2(1):99–120 doi:10.1146/annurev.anchem.1.031207.113005

(https://www.annualreviews.org/doi/abs/10.1146/annurev.anchem.1.031207.113005).

The Cochrane Collaboration (2011). Cochrane handbook for systematic reviews of interventions. The Cochrane Collaboration, Version 510

(https://handbook-5-1.cochrane.org/).

Thorntion MJ, Fritz JS, Klampfl CW (1997). Separation of native amino acids at low pH by capillary electrophoresis. J High Resolut Chrom. 20(12):647–52 doi:10.1002/jhrc.1240201206

(https://onlinelibrary.wiley.com/doi/abs/10.1002/jhrc.1240201206).

Thorsén G, Bergquist J (2000). Chiral separation of amino acids in biological fluids by micellar electrokinetic chromatography with laser-induced fluorescence detection. J Chromatogr B: Biomed Sci App. 745(2):389–97

(https://www.sciencedirect.com/science/article/pii/S0378434700003108).

Tkachuk R (1966a). Amino acid composition of wheat flours. Cereal Chem. 43(2):207–23.

Tkachuk R (1966b). Note on nitrogen-to-protein conversion factor for wheat flour. Cereal Chem. 43(2):223–5

(https://www.cabdirect.org/cabdirect/abstract/19661405777).

Tkachuk R (1969). Nitrogen-to-protein conversion factors for cereals and oilseed meals. Cereal Chem. 46(4):419–23

(http://agris.fao.org/agris-search/search.do?recordID=US201301210703).

Tkachuk R, Mellish VJ (1977). Amino-acid and proximate analyses of weed seeds. Can J Plant Sci. 57(1):243–9 doi:10.4141/cjps77-033

(https://www.nrcresearchpress.com/doi/pdfplus/10.4141/cjps77-033).

Todd JM, Marable NL, Kehrberg NL (1984). Methionine sulfoxide determination after alkaline hydrolysis of amino acid mixtures, model protein systems, soy products and infant formulas. J Food Sci. 49:1547–51

(https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2621.1984.tb12841.x).

Tomotake H, Shimaoka I, Kayashita J, Nakajoh M, Kat N (2001). Stronger suppression of plasma cholesterol and enhancement of the fecal excretion of steroids by a buckwheat protein product than by a soy protein isolate in rats fed on a cholesterol-free diet. Biosci Biotechnol Biochem. 65(6):1412–4 doi:10.1271/bbb.65.1412

(https://www.tandfonline.com/doi/abs/10.1271/bbb.65.1412).

Torbatinejad NM, Rutherfurd SM, Moughan PJ (2005). Total and reactive lysine contents in selected cereal-based food products. J Agric Food Chem. 53:4454–8

(https://pubs.acs.org/doi/10.1021/jf050071n).

Tsao M, Otter DE (1999). Quantification of glutamine in proteins and peptides using enzymatic hydrolysis and reverse-phase high-performance liquid chromatography. Anal Biochem. 269(1):143–8 doi:10.1006/abio.1998.3091

(https://www.sciencedirect.com/science/article/pii/S0003269798930913?via%3Dihub).

Van Boekel MAJS, Dumas BR (1987). Addendum to the evaluation of the Kjeldahl factor for conversion of the nitrogen content of milk and milk products to protein content. Neth Milk Dairy J. 41:281–4.

Vigo MS, Malec LS, Gomez RG, Llosa RA (1992). Spectrophotometric assay using o-phthaldialdehyde for determination of reactive lysine in dairy products. Food Chem. 44(5):363–5

(https://www.sciencedirect.com/science/article/pii/0308814692902698).

Villas-Bôas SG, Smart KF, Sivakumaran S, Lane GA (2011). Alkylation or silylation for analysis of amino and non-amino organic acids by GC-MS? Metabolites. 1(1):3–20 doi:10.3390/metabo1010003

(https://www.mdpi.com/2218-1989/1/1/3).

Voeten RLC, Ventouri IK, Haselberg R, Somsen GW (2018). Capillary electrophoresis: trends and recent advances. Anal Chem. 90(3):1464–81 doi:10.1021/acs.analchem.8b00015

(https://doi.org/10.1021/acs.analchem.8b00015).

Walker JM (2002). The bicinchoninic acid (BCA) assay for protein quantitation. In: Walker JM, editor. The protein protocols handbook (2nd edition). Totowa, NJ: Humana Press, 11–4

(https://link.springer.com/protocol/10.1385/1-59259-169-8:11).

Wallace JM, Fox PF (1998). Rapid spectrophotometric and fluorimetric methods for monitoring nitrogenous (proteinaceous) compounds in cheese and cheese fractions: a review. Food Chem. 62(2):217–24 doi:10.1016/s0308-8146(97)00162-3

(https://www.sciencedirect.com/science/article/pii/S0308814697001623?via%3Dihub).

Wanklin AJ, Chapman ET (1874). Water-analysis: A practical treatise on the examination of potable water (use of Nessler reagent). London: J. Alfred Wanklin MRCS

(https://archive.org/details/wateranalysisap01chapgoog/page/n41?q=%28Nessler+reagent%29+AND+date%3A%5B1850-01-01+TO+1860-01-01%5D).

Warburg O, Christian W (1942). Isolation and crystallization of enolase. Biochem Z. 310:384–421.

Watson ME, Galliher TL (2001). Comparison of Dumas and Kjeldahl methods with automatic analyzers on agricultural samples under routine rapid analysis conditions. Commun Soil Sci Plant Anal. 32(13–14):2007–19 doi:10.1081/CSS-120000265

(https://doi.org/10.1081/CSS-120000265).

Webb-Robertson B-J, Cannon WR (2007). Current trends in computational inference from mass spectrometry-based proteomics. Briefings in Bioinformatics. 8(5):304–17 doi:10.1093/bib/bbm023

(https://www.ncbi.nlm.nih.gov/pubmed/17584764).

Weiss M, Manneberg M, Juranville J-F, Lahm H-W, Fountoulakis M (1998). Effect of the hydrolysis method on the determination of the amino acid composition of proteins. J Chromatogr A. 795(2):263–75

(https://www.sciencedirect.com/science/article/pii/S0021967397009837).

Wetzel DL (1983). Near-infrared reflectance analysis. Anal Chem. 55(12):1165A–76A doi:10.1021/ac00262a001

(https://pubs.acs.org/doi/abs/10.1021/ac00262a001).

Whiffen LK, Midgley DJ, McGee PA (2007). Polyphenolic compounds interfere with quantification of protein in soil extracts using the Bradford method. Soil Biol Biochem. 39(2):691–4

(https://www.sciencedirect.com/science/article/pii/S0038071706003701).

Whitesell T, Aalhus JL, Larsen IL, Juárez M (2014). Evaluation of a rapid protein analyzer as a research tool for lean beef composition: effects of storage time and freezing. J Food Compos Anal. 33(1):67–70

(https://www.sciencedirect.com/science/article/pii/S0889157513001890).

WHO (2013). WHO recommendations on postnatal care of the mother and newborn. Geneva: World Health Organization (WHO)

(https://www.who.int/maternal_child_adolescent/documents/postnatal-care-recommendations/en/).

Wiercigroch E, Szafraniec E, Czamara K, Pacia MZ, Majzner K, Kochan K et al. (2017). Raman and infrared spectroscopy of carbohydrates: a review. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 185:317–35

(https://www.sciencedirect.com/science/article/pii/S1386142517304213).

Wilcox PE (1967). Determination of amide residues by chemical analysis. In: Hirs CHW, editor. Method Enzymol. New York: Academic Press

(https://www.sciencedirect.com/science/article/pii/S0076687967110094).

Yeoh H-H, Truong V-D (1996a). Amino acid composition and nitrogen-to-protein conversion factors for sweet potato. Trop Sci. 36(4):243–6.

Yeoh H-H, Truong V-D (1996b). Protein contents, amino acid compositions and nitrogen-to-protein conversion factors for cassava roots. JSci Food Ag. 70(1):51–4 doi:10.1002/(sici)1097-0010(199601)70:1<51::Aid-jsfa463>3.0.Co;2-w

(https://onlinelibrary.wiley.com/doi/abs/10.1002/%28SICI%291097-0010%28199601%2970%3A1%3C51%3A%3AAID-JSFA463%3E3.0.CO%3B2-W).

Yeoh H-H, Wee Y-C (1994). Leaf protein contents and nitrogen-to-protein conversion factors for 90 plant-species. Food Chem. 49(3):245–50 doi:10.1016/0308-8146(94)90167-8

(https://www.sciencedirect.com/science/article/pii/0308814694901678).

Yin B, Li T, Zhang S, Li Z, He P (2016). Sensitive analysis of 33 free amino acids in serum, milk, and muscle by ultra-high performance liquid chromatography-quadrupole-orbitrap high resolution mass spectrometry. Food Anal Methods. 9(10):2814–23 doi:10.1007/s12161-016-0463-0

(https://link.springer.com/article/10.1007%2Fs12161-016-0463-0).

You WW, Haugland RP, Ran DK, Haugland RP (1997). 3-(4-CarboxyBenzyl) quinoline-2-carboxaldehyde, a reagent with broad dynamic range for the assay of proteins and lipoproteins in solution. Anal Biochem. 244(2):277–82

(https://www.sciencedirect.com/science/article/pii/S0003269796999200?via%3Dihub).

Zándoki R, Csapó J, Csapó-Kiss Z, Tábori I, Domokos Z, Szűcs E (2006). Change of amino acid profile in Charolais cows' colostrum and transient milk during the first week post partum. Czech J Anim Sci. 51(9):375–82.

Zarkadas CG, Gagnon C, Gleddie S, Khanizadeh S, Cober ER, Guillemette RJD (2007). Assessment of the protein quality of fourteen soybean [Glycine max (L.) Merr.] cultivars using amino acid analysis and two-dimensional electrophoresis. Food Res Int. 40(1):129–46 doi:10.1016/j.foodres.2006.08.006

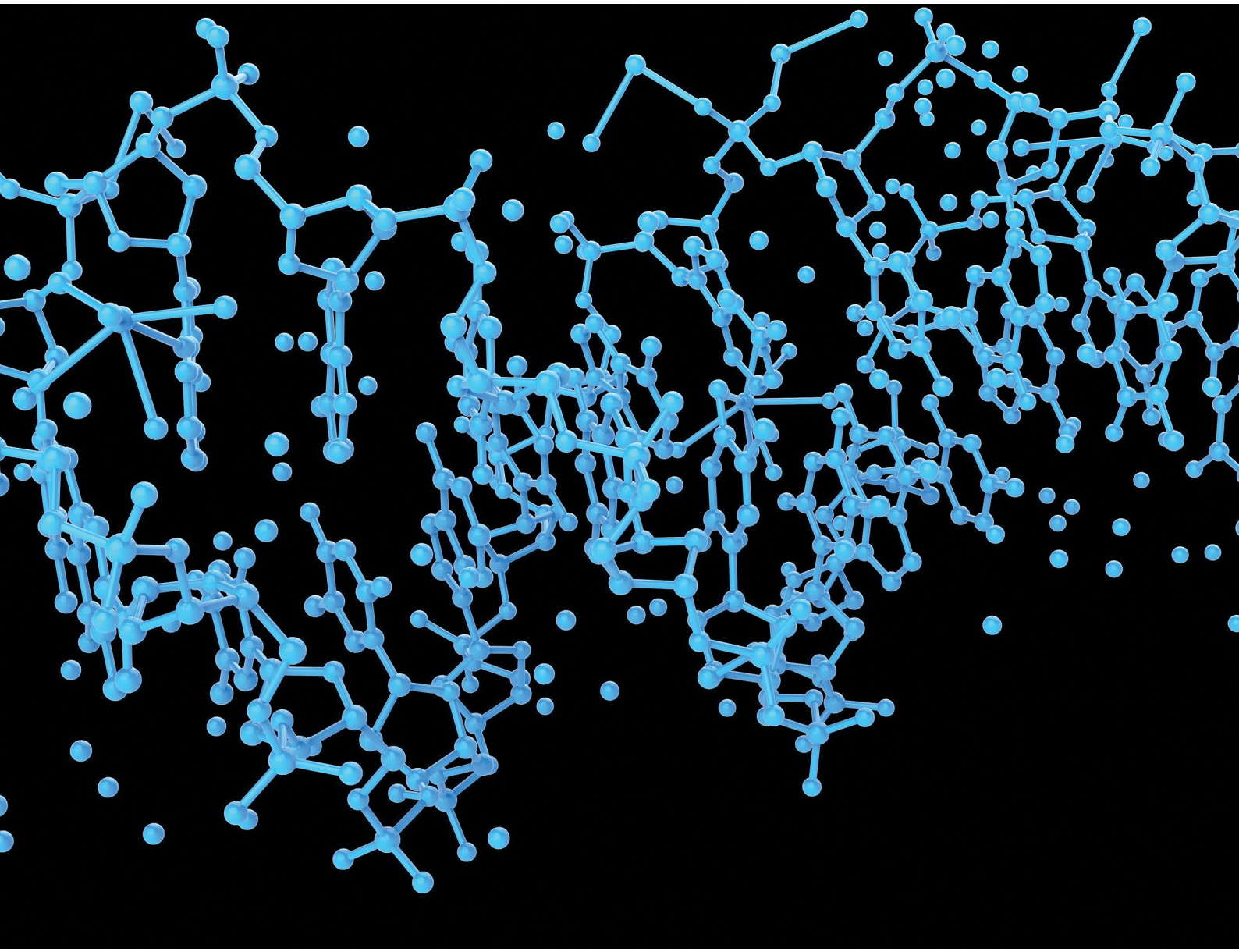
(https://www.sciencedirect.com/science/article/pii/S0963996906001311?via%3Dihub).

Zarkadas CG, Voldeng HD, Yu ZR, Choi VK (1997). Determination of the protein quality of three new northern adapted cultivars of common and miso type soybeans by amino acid analysis. J Agric Food Chem. 45(4):1161–8 doi:10.1021/jf9604201

(https://pubs.acs.org/doi/pdfplus/10.1021/jf9604201).

Zhu Z, Lu JJ, Liu S (2012). Protein separation by capillary gel electrophoresis: a review. Anal Chim Acta. 709:21–31

(https://www.sciencedirect.com/science/article/pii/S0003267011013328).



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