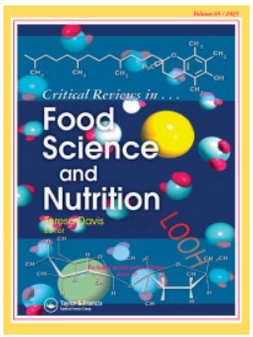

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REVIEW ARTICLE

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Toward harmonizing protein data in food composition databases: evaluating perspectives, methods and implications

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ABSTRACT

Protein content in foods has historically been estimated by multiplying measured nitrogen content with a universal nitrogen-to-protein conversion factor (NCF) of 6.25. Despite scientific consensus that this approach leads to systematic overestimations due to variations in amino acid composition and non-protein nitrogen (NPN) content, no universally accepted revision has been implemented. This review critically examines diverse perspectives on protein quantification and their implications for Food Composition Databases (FCDBs). A structured definition of protein for FCDBs is proposed, including amino acid residues, free amino acids and small peptides, while explicitly excluding NPN and prosthetic groups. Furthermore, analytical methods and NCF calculations are evaluated in order to provide more accurate assessments of protein content across a range of food matrices. The review highlights the importance of selecting food-specific NCFs to reduce overestimations, ensuring both scientific accuracy and practical feasibility. By addressing methodological shortcomings and proposing a refined protein quantification framework, this work aims to facilitate the transition toward more precise and harmonized protein values in FCDBs, benefiting nutritional research, dietary guidelines, and food labeling regulations.

KEYWORDS

amino acid residues; non-protein nitrogen; nitrogen-to-protein; conversion factor; NCF

1. History and background

The term protein was first introduced by Gerrit Jan Mulder in 1838, following a suggestion from the Swedish chemist Jöns Jacob Berzelius (Söderbaum 1916). Mulder initially determined protein content in food by measuring nitrogen, laying the foundation for future protein quantification methods (Mulder 1838, 1839). Later, many analytical methods to determine protein content in food and feed appeared. For decades, however, protein content has been primarily determined indirectly by measuring nitrogen content and using nitrogen conversion factors (NCF). Despite evolving analytical methods, the nitrogen measurement method developed by Johan Kjeldahl in 1883 (Kjeldahl 1883) remains widely used for protein determination.

Initially, a universal NCF of 6.25, based on Mulder's work, was used assuming that (1) proteins generally contain about 16% nitrogen and (2) all nitrogen in food is from proteins. However, many studies have shown these assumptions to be erroneous due to non-protein nitrogen (NPN) in foods, which contributes to measured nitrogen without impacting protein content (Mariotti, Tomé, and Mirand 2008). Moreover, amino acids have varying numbers of nitrogen atoms in side chains, causing the nitrogen content of proteins to range between 13% and 19%, depending on amino acid composition (Sáez-Plaza et al. 2013).

Despite these findings, and early discussions about the erroneous calculation of the protein content (Jones 1941), the NCF 6.25 is still widely used in food legislation, regulation, trade, economy, agriculture and science, as well as many food composition databases (FCDBs), leading to a significant overestimation of dietary protein content depending on the food group (Evers et al. 2016; Hayes 2020; Mæhre et al. 2018; Biancarosa et al. 2017). The continued use of 6.25 is partly due to the lack of standardized direct protein determination methods and the resource-intensive nature of other methods, such as amino acid analysis.

There is a recent trend toward plant-based diets and a growing emphasis on climate-friendly food options. As new protein sources such as seaweed, insects and legume-based

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meat analogs are introduced to meet this demand, the need for accurate protein data in general has increased. Standardizing protein determination is necessary to obtain accurate protein content values for foods, enabling the revision of protein data in FCDBs. This requires specifying protein components as well as reaching a consensus on their relevance to human nutrition.

Within the framework of EuroFIR AISBL (European Food Information Resource, *Association internationale sans but lucratif*), the protein component was re-assessed from the perspective of FCDB compilers and users, laying the groundwork for further investigation including the revision of FCDB protein data. EuroFIR is a nonprofit organization dedicated to promoting harmonization and correct use of high-quality nutrient data in Europe and beyond. It brings together food composition experts and FCDB compilers and users to create comprehensive food information resources (Durazzo et al. 2022).

In this review, we aim to establish a common understanding and terminology for protein specification, which will enhance the accuracy of protein data interpretation. This, in turn, will result in more precise responses to inquiries from stakeholders in nutrition research, the food industry, regulatory affairs, and sustainability.

2. Current perspectives on protein

Past attempts to define protein and its content in foods have often been tailored to specific scientific fields and based more on analytical methods than on biochemical structure and nutritional relevance (Moore et al. 2010). The different perspectives from various disciplines are briefly outlined below, followed by the definition of protein for FCDB (see section 3) with reference to the perspectives (Figure 1).

2.1. The chemical perspective of protein

Proteins are made of L-α-amino acids linked by peptide bonds. All amino acids contain a carboxyl and an amino group at the α-carbon atom, with variations occurring in the side chains including the nitrogen content. For example, proteins rich in the amino acids glutamine, asparagine, arginine, and lysine have higher nitrogen contents compared to proteins containing mostly other proteinogenic amino acids (Heinrich et al. 2022).

Peptide chains with several to many amino acids are called oligopeptides or polypeptides, while longer chains or molecules with multiple chains are referred to as proteins (Lopez and Mohiuddin 2024; Gaßmann 2006). Proteins have

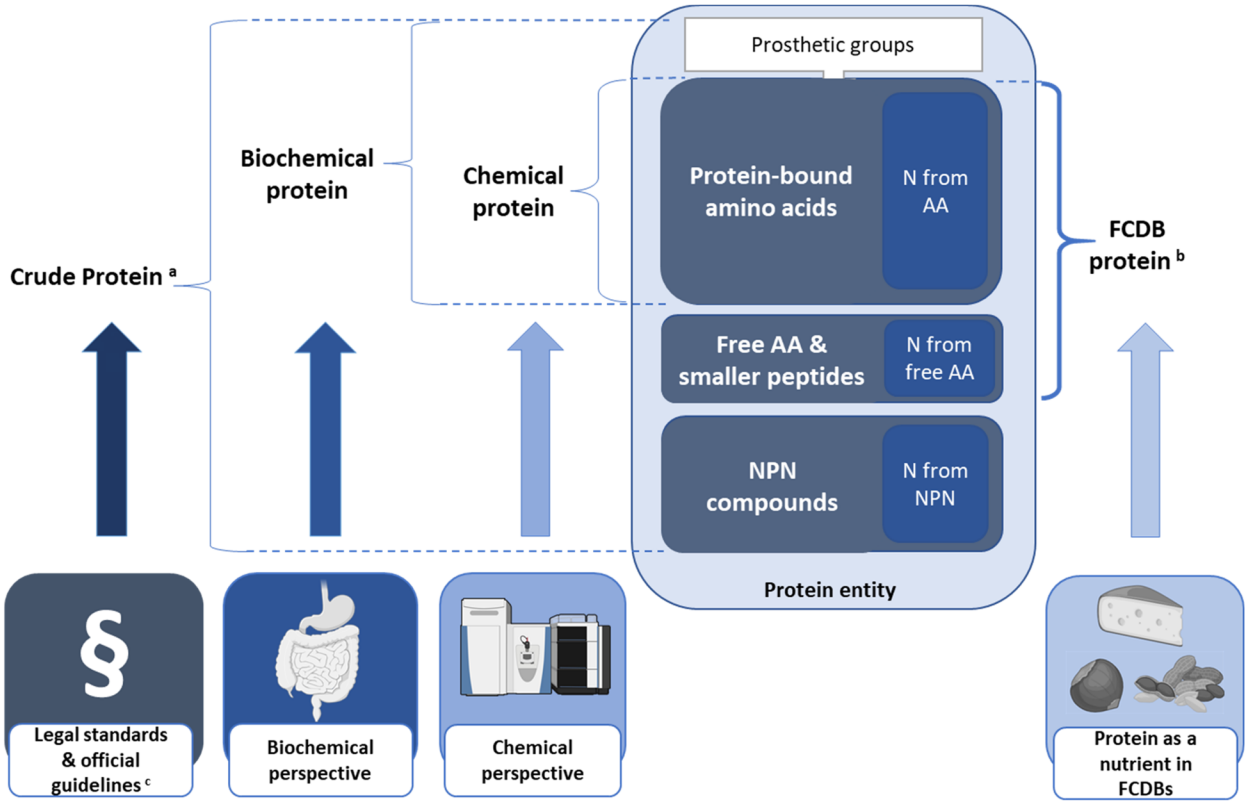

Figure 1. Overview of different perspectives on protein, including legal standards, biochemical, and chemical definitions, and the proposed revised definition for Food Composition Databases (FCDBs). Perspectives currently used in most FCDBs (e.g., crude protein calculation as $N \times 6.25$) are indicated, alongside the proposed refinements, which account for amino acid residues, free amino acids, and small peptides while excluding non-protein nitrogen (NPN) and prosthetic groups. AA: amino acids; N: nitrogen, NPN: non-protein nitrogen.

[a]Currently used in most Food Composition Databases (FCDBs), relying on crude protein (N × 6.25) without differentiation of NPN content.

[b]Proposed refinement for FCDBs, incorporating free amino acids and small peptides while excluding NPN and prosthetic groups.

[c]Legal and regulatory definitions continue to use crude protein calculations; however, updated nitrogen-to-protein conversion factors (NCFs) are recommended for more accurate protein content assessment.

a primary sequence and secondary, tertiary, and often quaternary structures. The tertiary structure, or three-dimensional (3D) shape, is crucial to biological function (Gaßmann 2006; Sanvictores and Farci 2022).

Originally, 20 amino acids were known to be proteinogenic, meaning they are incorporated into proteins during protein synthesis in living organisms. These amino acids are categorized as indispensable (IAA; formerly essential) and dispensable (DAA; formerly non-essential) amino acids. IAA must be obtained from the diet, since humans cannot synthesize them (Voet and Voet 2010; Heinrich et al. 2022). There is ongoing debate about whether cysteine and tyrosine can partially replace the IAA methionine and phenylalanine in humans (FAO 2013).

2.2. The biochemical perspective of protein

From a biochemical perspective, proteins are large, complex molecules made of amino acids linked by peptide bonds into polypeptide chains. These chains fold into specific 3D structures that determine function (Wu 2013).

Proteins (except for pathophysiological variants) are essential macromolecules with critical roles in living organisms. They form enzymes, enabling key metabolic processes such as digestion, molecule synthesis, cell regulation, and structural functions in muscles and skin (San Gabriel and Uneyama 2013). In addition, proteins often contain prosthetic groups - non-amino acid components that are essential for their function. These groups help regulate catalytic activity and contribute to structural integrity, solubility, and electrostatic properties.

Dietary proteins are complex macromolecules from animal or plant sources that provide amino acids for the human body. After ingestion, proteins undergo metabolism, starting with digestion in the stomach and small intestine. In the stomach, proteins undergo denaturation by hydrochloric acid, and the enzyme pepsin breaks down proteins into smaller peptides. In the small intestine, pancreatic enzymes, such as trypsin and chymotrypsin, further degrade these peptides into shorter chains and individual amino acids. These breakdown products are absorbed through the intestinal wall into the bloodstream and then transported to tissues and organs (Moughan 2016). Amino acids serve as building blocks for protein synthesis, enabling growth, repair, and maintenance of body tissues. In addition to their role in protein formation, some amino acids are converted into hormones (e.g., T3, T4, melatonin) or neurotransmitters (e.g., adrenaline, noradrenaline, dopamine). Excess amino acids can also be utilized for energy production or converted into other molecules (FAO, WHO, and UNU 2007).

2.3. Protein from the perspective of legal standards and official guidelines

While chemical and biochemical aspects of protein are well understood, many authoritative documents focus primarily on protein determination methods for purposes like nutrition labeling. As a result, protein is often described as the nitrogen content of food multiplied by the NCF 6.25, and commonly referred to as crude protein (see Table 1).

Exclusive reliance on nitrogen content to describe protein content in legal standards and official guidelines without accounting for NPN and key chemical and biochemical insights is understandable, but concerning. It is significant because legal documents influence the global use and interpretation of protein content across regulatory, science, and food industry sectors.

3. The FCDB perspective of protein as a nutrient

There is still confusion among users about interpreting protein content from reference works like FCDBs and its implications. While proteins are generally understood as polypeptide chains in chemical and biochemical contexts, FCDBs often focus on crude protein, which conflicts with this understanding. When describing protein as a nutrient in FCDBs, it is crucial to contemplate its role and applications to ensure clear and consistent use of the term 'protein' for both compilers and users.

3.1. The role of protein in FCDBs

FCDBs provide comprehensive information on food composition, mainly focusing on nutrients but also including other bioactive molecules and contaminants (Greenfield and Southgate 2003). They are essential for nutritionists, dietitians, researchers, and food industry professionals, and have a crucial role in dietary assessment, nutritional research, food labeling, and the development of dietary guidelines and public health policies. Among the various components, protein content is specifically measured for labeling, regulatory compliance, economic valuation, and quality control of protein-rich foods (Sáez-Plaza et al. 2013; Moore et al. 2010).

The term 'protein' can be ambiguous due to its various uses. If the purpose is to simply describe the nitrogen content in food, a general NCF can persist. When focusing on the provision of dietary amino acids, the protein component must reflect amino acid composition using specific amino acid data or updated NCFs (Mariotti, Tomé, and Mirand 2008; Moore et al. 2010). Nutritionally, the main role of protein is to supply essential amino acids and nitrogen, with biochemical traits being secondary (Moore et al. 2010; Morgan et al. 2023).

3.2. Characteristics of protein in FCDBs

With the above-mentioned role of protein in mind, protein as a nutrient in FCDBs can be further characterized.

3.2.1. Free amino acids and smaller peptides

Although the chemical and biochemical understanding of proteins exclude free amino acids and smaller peptides, there is an ongoing debate about their inclusion in the protein

Table 1. Summary of current authoritative protein descriptions.

Authoritative document	Description	Reference
Code of Federal Regulations on Food Labeling in the US	"Protein content may be calculated on the basis of the factor 6.25 times the nitrogen content of the food as determined by the appropriate method of analysis as given in the Official Methods of Analysis of the AOAC International, except when official AOAC procedures described in this paragraph (c) (7) require a specific factor other than 6.25, that specific factor shall be used"	Code of Federal Regulations (2024)
EFSA NDA Panel	"Proteins are built from amino acids joined together by peptide bonds […] Twenty of the naturally occurring amino acids are so-called proteinogenic amino acids which build proteins in living organisms. […] A conversion factor of 6.25 is usually used for the conversion of nitrogen to protein for labeling purposes, assessment of protein intake, and for protein reference values. Total N x 6.25 is called crude protein and [total minus non-protein-N] x 6.25 is called true protein… In this opinion, unless specifically mentioned, 'protein' is total N x 6.25 and protein requirements are calculated from nitrogen content"	EFSA Panel on Dietetic Products Nutrition and Allergies (2012)
FAO Food and Nutrition Paper 77: Food energy - methods of analysis and conversion factors	"Proteins are large molecules made of amino acids." [Analytical methods:] "Preferred: Protein is best measured as the sum of individual amino acid residues. Acceptable: Until values for protein based on amino acid analyses are generally available, protein based on total nitrogen (N) by Kjeldahl (or comparable method) x a factor is acceptable."	FAO (2003)
Guidelines on Nutrition Labeling (rev 2021)	"3.3.2 Calculation of protein: The amount of protein to be listed should be calculated using the formula: Protein = Total Kjeldahl Nitrogen x 6.25 unless a different factor is given in a Codex standard or in the Codex method of analysis for that food."	FAO and WHO (1985)
National Food Safety Standard of the P.R.C.: General Rules for Nutrition Labeling of Prepackaged Foods (GB28050) in China	"2.5.1 Protein: Nitrogen-containing organic compounds in food, with amino acids as the basic unit. The protein content in food can be determined by multiplying the total nitrogen content by the protein conversion coefficient, or the sum of the amino acid content in the food"	Ministry of Health of the PRC (2021)
Regulation (EU) No 1169/2011	"Protein means the protein content calculated using the formula: protein = total Kjeldahl nitrogen × 6.25". The definition was identical to that in the now-repealed Council Directive 90/496/EEC of 24 September 1990 on nutrition labeling for foodstuffs	Official Journal of the European Union (2011)
Resolução – RDC in Brazil	"2.8. Proteins are polymers of amino acids or compounds containing polymers of amino acids"	Agência Nacional de Vigilância Sanitária (2003)

component due to their role in the human body (FAO and WHO 2019).

Nutritionally, free amino acids and smaller peptides should be considered part of the protein definition, as they deliver amino acids to the body (Greenfield and Southgate 2003; Sosulski and Imafidon 1990; Moore et al. 2010; Mariotti, Tomé, and Mirand 2008; Misra 2001). Recent studies suggest that free (indispensable) amino acids, such as those used in food fortification, might enhance plasma amino acid concentrations and stimulate muscle protein synthesis more effectively than the same amount delivered as intact proteins (Ferrando et al. 2023; Weijzen et al. 2022).

While in the average diet free amino acids contribute less to total protein intake compared with intact protein, they are still valuable for protein synthesis and other metabolic processes. They can be particularly important in situations where protein digestion or absorption is impaired (Tomé 2022; Churchward-Venne et al. 2013).

3.2.2. Non-protein nitrogen (NPN) components

Total nitrogen in food includes both protein nitrogen and NPN, which are both measured by standard methods such as Kjeldahl or Dumas. When the measured nitrogen content is multiplied by an NCF of 6.25, the true protein content is therefore overestimated. NPN concentrations vary widely depending on food type, production process, and protein purification rate (Mariotti, Tomé, and Mirand 2008), causing significant error in standard analyses of food protein content (Mossé 1990; Mariotti, Tomé, and Mirand 2008; WHO and FAO 2019; Jones 1941; Periago et al. 1996). NPN is especially high in plant-based foods (Maubois and Lorient 2016; Periago et al. 1996; Imafidon and Sosulski 1990), but also significant in fish and meat products (Salo-Väänänen and Koivistoinen 1996) (see Supplementary Table S1 for examples of NPN amounts in different food items).

The most common NPN compounds include non-proteinogenic amino acids, nitrate/nitrite, ammonia and ammonium compounds, creatine/creatinine, nucleotide bases, vitamins, alkaloids, amines, phospholipids, and nitrogenous glycosides. Most of these compounds are not α-amino acids, but some publications include free amino acids and smaller peptides in the definition of NPN (Maubois and Lorient 2016; Imafidon and Sosulski 1990; Sosulski and Imafidon 1990).

Non-proteinogenic amino acids can also occur in proteins. For example, 4-hydroxyproline, found in connective tissues like collagen, is not directly incorporated into proteins during translation and is, instead, formed through post-translational modification of proline (Hu, He, and Wu 2022). Despite its potential role in metabolism, collagen synthesis, and antioxidant activity, it is not a standard proteinogenic amino acid (Hu, He, and Wu 2022; Wu et al. 2011) and, therefore, should be excluded from the protein definition until further research proves otherwise.

There are other non-proteinogenic amino acids found in protein, but to a much lesser extent. For example, selenocysteine naturally occurs in certain enzymes, the abundance of which in food is very low (Johansson, Gafvelin, and Arnér 2005). The same is true for pyrrolysine, which can be found

in enzymes involved in methanogenesis and archaea but is not commonly found in food proteins (Brugère et al. 2018). γ-aminobutyric acid (GABA) is a non-proteinogenic amino acid and common neurotransmitter in the central nervous system, but not typically found in proteins either (Ramos-Ruiz, Martinez, and Knauf-Beiter 2019). In contrast, certain other amino acids can occasionally be present in food proteins. For example, norleucine and norvaline (analogs of leucine and valine) can accidentally be incorporated into proteins during translation (Anderhuber et al. 2016). β-amino acids occur naturally only in bacteria and invertebrates (Lelais and Seebach 2004), while hydroxylysine, similar to 4-hydroxyproline, is found in collagen, but less frequently (Myllyharju 2005). However, due to their rare occurrence and lack of routine analysis, these amino acids should not be considered part of the protein component.

During food processing, particularly under heat (e.g., protein extrusion) and alkaline conditions, various process-induced non-proteinogenic amino acids, such as lysinoalanine (LAL), methionine-sulfone, carboxymethyl-lysine, furosine, and D-amino acids can form (Schweiggert-Weisz et al. 2024). LAL is a cross-link product formed from lysine-residues and dehydroalanine through the Maillard reaction (McKerchar et al. 2023), while D-amino acids result from the racemization of L-amino acids during peptide bond cleavage at high temperatures (Friedman 1999). Although these process-induced non-proteinogenic amino acids are present in low concentrations and therefore do not constitute part of the protein component, they can decrease protein digestibility and bioavailability in terms of protein quality (Meade, Reid, and Gerrard 2005), which is covered in section 6.4.

3.2.3. Prosthetic groups

A prosthetic group is a non-amino acid moiety tightly bound to a protein, often covalently, in a heteroprotein or conjugated protein. These groups have an important role in enzyme catalysis, act as cofactors or coenzymes, serve as electron carriers, or help maintain protein structure. A protein without its prosthetic group is called an apoprotein, while one with its prosthetic group is called a holoprotein or heteroprotein. As explained in section 2.2, prosthetic groups are essential for biological function, as apoproteins cannot perform their role without them (Maubois and Lorient 2016; Evers et al. 2016).

From a nutritional standpoint, prosthetic groups do not contribute to the definition of dietary protein since they do not provide amino acids. The dairy industry advocates for including prosthetic groups in protein, resulting in higher NCFs compared with excluding them (Krul 2019).

Analytical determination of prosthetic groups for every food item is not feasible, and extensive knowledge about prosthetic groups is only available for a few foods, such as dairy proteins and milk (Maubois and Lorient 2016; FAO and WHO 2019). As prosthetic groups increase the mass of isolated protein in analytical measurements of food protein content, their weights must be considered in protein determination methods and calculation of conversion factors (FAO and WHO 2019).

Summary section 3 (see also Figure 1):

- From a nutritional perspective, free amino acids and smaller peptides should be included in the protein component for FCDBs.
- NPN should not be considered part of protein as a nutrient, provided that small peptides and free proteinogenic amino acids are classified under the protein fraction, not the NPN fraction.
- Other amino acids found in protein structures (e.g. 4-hydroxyproline, selenocysteine, pyrrolysine, GABA, norleucine/norvaline, hydroxylysine) should not be part of the protein component.
- Prosthetic groups are not a direct part of the protein component.
- The presence of prosthetic groups might be considered in NCF calculation, depending on the chosen type of NCF (see 5.2).

4. Analytical methods for protein determination

Various methods exist to determine protein content in foods, each with different principles, methodologies, advantages, and disadvantages (Table 2). The selection of an analytical method typically depends on criteria such as ease of use, accuracy, cost-effectiveness, and the alignment of the measured variable with the intended purpose. These methods focus on different aspects of protein structure, making the choice highly dependent on the target application (Chang 2017). Therefore, evaluating the suitability of the methodologies for protein quantification is essential for understanding protein in FCDB.

The most accurate method for assessing protein content regarding the FCDB definition of protein is amino acid analysis, which aligns with the nutritional role of protein to deliver essential amino acids and peptides. Other approaches, such as the Biuret method, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and near-infrared (NIR) spectroscopy, focus on peptide bonds, specific side chains, or molecular vibrations. Quantification via these methods depends on amino acid composition, especially when standards like bovine serum albumin (BSA) or casein differ from the investigated food matrix (Kamizake et al. 2003). In nitrogen determination, using specific NCFs can account for differences in amino acid composition across different food matrices, ensuring more accurate protein quantification (Sáez-Plaza et al. 2013).

The preferred methods to determine protein content of foods for use in FCDBs, which align with FAO recommendations for protein analysis (Maclean et al. 2003), are:

1. Direct analysis of amino acids
2. Determination based on total (organically bound) nitrogen by Kjeldahl or Dumas methods, provided that suitable nitrogen-to-protein conversion factors are available for specific food matrices.

Secondary methods like NIR should only be used if calibrated with the preferred methods (Moore et al. 2010; Chang 2017; Lynch and Barbano 1999). Newer biochemical methods like enzyme-linked immunosorbent assay (ELISA), liquid chromatography-mass spectrometry (LC-MS), or capillary electrophoresis might selectively measure protein content, but they have not been thoroughly tested for routine application in different food matrices (Moore et al. 2010).

Table 2. Overview of available analytical methods for protein determination in foods.

Method	Principle	Measured protein functionality	Advantages/disadvantages	References
Amino acid analysis	Hydrolysis of the protein and quantification of amino acids by chromatography	Direct determination of amino acids as building blocks of protein	+ direct determination of true protein - time-consuming and expensive; underestimation due to poor recovery in hydrolysis	Rutherfurd and Dunn (2011); Otter (2012)
Kjeldahl method	Acid digestion of the food sample to convert all organically bound nitrogen into ammonia which is then quantified titrimetrically	Occurrence of organically bound nitrogen in the sample	+ easy to use; standardized and therefore comparable between laboratories - not specific and prone to overestimation due to other nitrogenous compounds in the sample	Kjeldahl (1883); Sáez-Plaza et al. (2013)
Dumas method	Combustion of the sample to convert all available nitrogen into nitrogen gas	Occurrence of total nitrogen in the sample	+ fast; no chemicals involved - not specific and prone to overestimation due to other nitrogenous compounds in the sample	Dumas (1831)
Bradford assay	Binding of Coomassie brilliant blue dye to proteins	Dye binds non-covalently to the carboxyl and amino groups of the protein → interaction of the dye and proteins	+ less prone to interferences than Biuret/Lowry - reaction varies with AA composition; small peptides and free AA are not detected	Bradford (1976); Kamizake et al. (2003)
Biuret method	Biuret reagent reacts with peptide bonds in proteins leading to a color change	Reaction between the peptide bond and copper(II)-ions; especially by reducing amino acids like cysteine or tyrosine residues → measurements of peptides	+ simple method - unspecific reaction, two major modifications available (Lowry and BCA assay), prone to interferences	Torten and Whitaker (1964); Kamizake et al. (2003)
Lowry method	Reaction of the protein with copper under alkaline conditions followed by reaction with Folin Ciocalteu reagent resulting in a blue complex	Reaction between the peptide bond and copper(II)-ions; especially by reducing amino acids like cysteine or tyrosine residues → proportions of the amino acids responsible for the effect influence the effect intensity	+ more sensitive than Biuret method - time consuming, prone to interferences, more suitable for clinical samples; food matrices require extensive sample preparation	Hartree (1972); Lowry et al. (1951)
Near infrared spectroscopy (NIR)	Measurement of the NIR absorption by the sample. Resulting fingerprint pattern	Different overtones and combination vibrations of functional groups in molecules produce signals in the NIR; after calibration, the signals can be used for protein quantification	+ nondestructive; fast; for routine analysis - needs elaborate calibration for each food matrix	Kays, Barton, and Windham (2000); Ingle et al. (2016)
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)	After electrophoresis which separates proteins by their mass, the proteins are visualized (with or without dyes). Intensity of visualization corresponds to the quantity of protein	Visualization uses different functionalities in the protein entity, e.g., Coomassie brilliant blue attaches to the basic side chains of the amino acids → proportions of the amino acids responsible for the effect influence the effect intensity	+ good combination with information about protein fractions - only semi-quantitative; time-consuming; different effects of proteins	Holzmüller and Kulozik (2016)

AA, amino acids; BCA, bicinchoninic acid.

Summary section 3:
- Direct analysis of amino acids should be the preferred analytical method for protein determination
- Alternatively, total (organically bound) nitrogen by Kjeldahl or Dumas methods can be analyzed to determine protein content, provided suitable NCFs are available for the specific food matrices.

5. Protein calculation

The preferred analytical methods do not deliver protein content of a sample directly: protein quantity must be calculated based on the measured parameter. The choice of the calculation method is crucial.

5.1. Calculation of protein from amino acid data

Two approaches exist for using amino acid analysis in the calculation of protein content. The first considers each amino acid as a whole and sums amino acid masses into protein according to Yeoh and Truong (1996):

$$Prot\left[\frac{g}{100g}\right]=\sum_{i=1}^{18}\left(\mathrm{AA}_i\left[\frac{g}{100g}\right]\right)$$

It should be noted that this approach does not consider water introduced during hydrolysis and, therefore, overestimates protein content. Thus, accurate protein calculations from amino acid data must use amino acid residues, not the total mass of the amino acids, to avoid overestimating protein content, especially in longer polypeptide chains (FAO and WHO 2019). The resultant true protein content is the sum of all 18 quantifiable proteinogenic amino acid residues:

$$Prot\left[\frac{g}{100g}\right]=\sum_{i=1}^{18}\left(\mathrm{AAR}_i\left[\frac{g}{100g}\right]\right)$$

Notably, asparagine and glutamine are converted to aspartic and glutamic acids, respectively, during the hydrolysis step of amino acid determination (Boulos, Tännler, and Nyström

2020). Since the difference in molecular weight of the acidic to amine form is only 1 g/mol, error in the summation of total amino acid residues using only the acidic forms of Glx und Asx is considered negligible (Krul 2019).

5.2. Calculation of protein from nitrogen data with NCF

The second preferred method for protein determination involves multiplying total (organically bound) nitrogen in a food sample by a conversion factor that accounts for differences in the amino acid composition and NPN content. Natural variation in NPN can lead to differences in the resulting NCF (Moore et al. 2010; Estrella 2008). Key publications addressing the challenges and discrepancies in calculating accurate NCFs include works by Mossé (1990); Mariotti, Tomé, and Mirand (2008); Krul (2019); FAO and WHO (2019).

Some studies have published specific NCFs for foods (Fujihara, Kasuga, and Aoyagi 2001; Fujihara et al. 1995; Fujihara et al. 2008; Fujihara, Sasaki, and Sugahara 2010; Mossé 1990; Morr 1982; Sosulski and Imafidon 1990; Tkachuk 1969; Yeoh and Truong 1996), but these NCFs are often defined differently, resulting in various NCF types (see Table 3).

For determining the total nitrogen content of a sample, two primary methods are available: the Kjeldahl method, which measures total organically bound nitrogen, and the Dumas combustion method, which determines total nitrogen. Differences may occur between these methods, with the Dumas method giving approximately 1.5% higher nitrogen values than the Kjeldahl method, particularly in samples with high concentrations of inorganic nitrogen or organic compounds resistant to acid digestion (Jung et al. 2003; Müller 2017; Thompson et al. 2002; Simonne et al. 1997). However, the bias between methods can vary significantly, both between types of food and between individual examples of a particular type, with fish-based products showing the highest bias of 2% and meat-based products showing the lowest of 0.7%. Furthermore, there may be a variation in bias with the concentration of the analyte (Thompson et al. 2002). While such variations exist, they are generally not substantial enough to justify separate NCF calculations based on the chosen analytical method for nitrogen.

Based on inclusion or exclusion of various substance groups, the following decisions about NCF selection can be made:

- $k_{6.25}$ and Jones factors: Due to shortcomings in determining these factors, they should be excluded
- Prosthetic groups: Exclusion of prosthetic groups thus leads to the exclusion of factor K', which is based on the inclusion of prosthetic groups.

Table 3. Overview of nitrogen-to-protein conversion factor (NCF) calculation methods in the scientific literature.

Abbreviation	Abbreviation from reference	Equation	Description	Reference
$k_{6.25}$	-	$=\frac{100\%}{16\%\ nitrogen\ in\ protein\ fraction}$	Based on the assumption, that proteins in general contain around 16% nitrogen	Mulder (1839); Official Journal of the European Union (2011)
k_{Jones}	-	$=\frac{100\%}{x\%\ nitrogen\ in\ protein\ fraction}$	Based on the nitrogen content of extracted proteins in the sample	Jones (1941)
k_{AA}	k_A	$=\frac{\sum_{i=1}^{18}(AA_i)}{N_{AA}}$	Ratio of protein (sum of amino acids AA) to nitrogen from amino acids N_{AA} but excluding the amide-N	Yeoh and Truong (1996)
k_A	k_A=K_A	$=\frac{\sum_{i=1}^{18}(AAR_i)}{N_{AA}+N_{amide}}$	Sum of amino acid residues AAR to amino acid nitrogen N_{AA} from all 20 proteinogenic amino acids (including amide nitrogen N_{amide} from Asn and Gln; "pure protein factor")	Mossé (1990); FAO and WHO (2019)
k_A'	k_A'	$=\frac{\sum_{i=1}^{18}(AAR_i)}{N_{AA}+N_{hydrolysis\,NH3}}$	See k_A, but N_{amide} is replaced by NH_3 from hydrolysis (with 6 M HCl) as a proxy	Mossé (1990); FAO and WHO (2019)
k_P	k_P=K_B	$=\frac{\sum_{i=1}^{18}(AAR_i)}{N_{total}}$	Sum of amino acid residues AAR to total nitrogen N_{total} by Kjeldahl or Dumas	Mossé (1990); FAO and WHO (2019)
k_N	k_N	$=\frac{\left(\sum_{i=1}^{18}(AAR_i)\right)+\sum(nucleic\ acids)}{N_{total}}$	Main nitrogen containing compounds (sum of amino acid residues AAR plus sum of nucleic acids) to total nitrogen N_{total}	Mossé (1990)
k	k	$=\frac{k_P+k_A}{2}$	True conversion factor as the average of k_P and k_A	Mossé (1990)
K'	K or K'	$=\frac{polypeptides\ incl.prosthetic\ groups}{N_{total}\ or\ N_{Protein}}$	Based on the amino acid composition/sequence of the proteins in the source, including prosthetic groups in the polypeptide mass Sum of amino acid residues plus the sum of determined prosthetic groups to (protein) nitrogen	Mariotti, Tomé, and Mirand (2008); FAO and WHO (2019)
K	K' or K	$=\frac{polypeptides\ excl.prosthetic\ groups}{N_{total}\ or\ N_{Protein}}$	Based on the amino acid composition/sequence of the proteins in the source, excluding prosthetic groups in the polypeptide mass	Mariotti, Tomé, and Mirand (2008); FAO and WHO (2019)

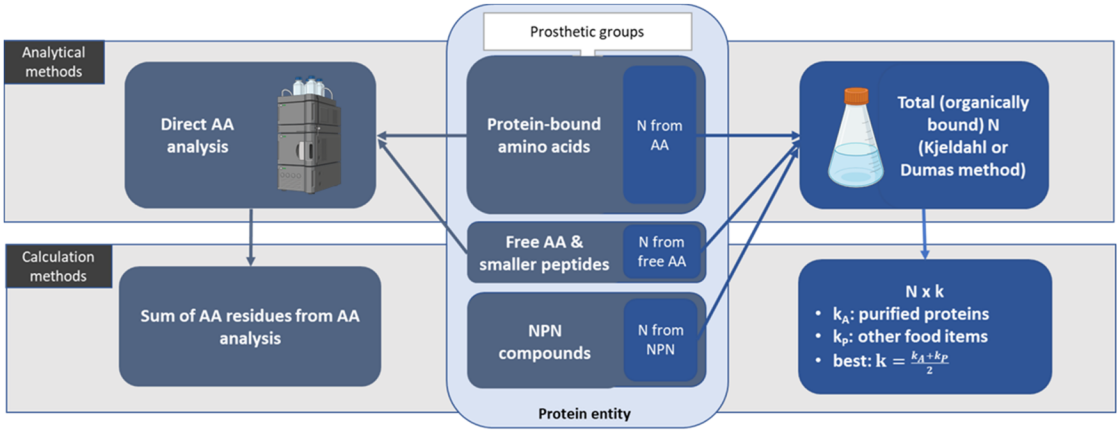

Figure 2. Overview of the preferred analytical and calculation methods for determining protein content in Food Composition Databases (FCDBs). The two primary calculation methods include: (1) true protein content based on the sum of amino acid residues, and (2) protein estimation from total nitrogen content using a suitable nitrogen-to-protein conversion factor (NCF). AA: amino acids, N: nitrogen, NPN: non-protein nitrogen, k_A: NCF for purified protein, k_P: NCF for other food items (see Table 3).

- NPN fraction: Excluding NPN while including free amino acids and smaller peptides means excluding factor $k_{N,}$ which accounts for nucleic acids. This factor is of theoretical interest only (Mossé 1990).
- AA composition/sequence of the proteins: Practical difficulties in determining amino acid composition and ignoring NPN lead to the exclusion of K (polypeptide method (Mariotti, Tomé, and Mirand 2008))

NCF calculation methods should distinguish between protein isolates – purified protein fractions with low NPN content – by applying the NCF k_A, and other food items with higher NPN content by applying the NCF k_P. Ideally, the final NCF should be an average of both values – the factor k.

Summarizing sections 5.1 and 5.2, an overview of preferred analytical methods with subsequent calculation steps for protein determination is given in Figure 2.

Exemplary calculations for both methods, applied to bread wheat wholemeal flour, are provided in the Supplementary Material. The first method determines protein as the sum of amino acid residues (Supplementary Table S2), whereas the second method calculates protein using total (organically bound) nitrogen content and a specific NCF (e.g., 5.49 for wheat flour from Mariotti, Tomé, and Mirand (2008)).

Summary Section 5:
- Preferred methods: While amino acid data provides the most accurate protein content, nitrogen-based estimates are a resource-efficient alternative, requiring specific conversion factors.
- Amino Acid Data: Use the cal culation from amino acid residues, not whole AA, to avoid overestimating the protein content.
- Purified Protein Fractions: Use factor k_A for nitrogen-to-protein conversion. If k_A is not available, $k_{A'}$ can be used as an alternative.
- Other Food Items: Use factor k_P.
- True Conversion Factor: Assess and use the 'true conversion factor' k, which is an average of k_A and k_P, except for low-protein and high-NPN foods where k_P is more suitable.
- Legal Requirements: FCDBs can continue using $k_{6.25}$ factor to meet legal requirements for nutritional labeling, alongside the defined protein component.

6. Discussion

6.1. Characteristics of the proposed revised definition

Revising the protein component in FCDBs requires a clear definition to ensure consistency and clarify its intended use. The proposed protein definition aligns with the chemical and biochemical understanding of proteins, emphasizing their role in supplying IAA. This definition guides the selection of appropriate analytical and computational methods for protein determination, improving accuracy across food categories.

Unlike previous definitions, which often lacked alignment between theoretical principles and practical applications, particularly in legal and regulatory contexts, the revised approach incorporates free amino acids and smaller peptides while excluding NPN and prosthetic groups. However, prosthetic group should still be accounted for when determining NCFs. Preferred protein quantification methods include direct amino acid analysis and total nitrogen determination (via Kjeldahl or Dumas), using food-specific NCF. For amino acid-based protein content estimation, calculations based on amino acid residues prevent overestimation, while nitrogen-based estimates should employ factor k_A for purified protein fractions and k_P for general food matrices.

This refined protein definition establishes a global framework for quality control in nitrogen and amino acid analyses, ensuring greater consistency in protein assessment across food chemistry, nutrition science, and regulatory applications. By eliminating systematic overestimation in FCDBs, it enhances the comparability of protein values across diverse food sources, supporting more precise dietary assessments, food labeling, and industrial formulations.

6.2. Advantages and potential impacts on dietary assessments, food labeling, and economic considerations

6.2.1. Dietary assessment and nutrition research

Accurate protein values are essential for evaluating dietary protein intake, assessing nutritional adequacy, and developing evidence-based dietary guidelines. Current FCDB data tend to overestimate protein intake, leading to inflated

nutritional adequacy assessments. Implementing food-specific NCF or amino acid-based protein data would improve dietary models, particularly for populations relying on plant-based protein sources, where protein digestibility and amino acid composition vary significantly. More precise intake estimations could impact dietary recommendations and nutritional guidelines, ensuring they align more closely with true bioavailable protein intake.

6.2.2. Relevance for food labeling and regulatory compliance

Protein content declarations significantly influence consumer perception, regulatory compliance, and health-related food claims. Under current regulations, protein values are primarily calculated using $N \times 6.25$, disregarding variations in amino acid composition and NPN content. Implementing revised protein calculation including food-specific NCF would enhance labeling accuracy, ensuring protein content claims reflect actual nutritional contribution. However, transitioning to revised calculations may require updates to food labeling laws and threshold adjustments for high-protein food certifications.

6.2.3. Economic consequences of revising protein values

Since protein content directly influences the valuation of protein-rich ingredients, adjustments in protein calculation have economic implications for food producers, manufacturers, and trade regulations (Koletzko and Shamir 2006). Industries that rely on high-protein claims, such as dairy, meat, and plant-based protein manufacturers, may need to adapt to changes in reported protein content. In some cases, reformulating products to maintain protein content claims might be necessary. For plant-based protein sources, which often exhibit high overestimation due to high NPN content (see supplementary Table S1), the revised calculations may shift competitive dynamics in the protein market. At the same time, improving transparency in protein labeling could strengthen consumer trust and regulatory credibility, reinforcing long-term industry stability despite potential short-term disruptions during the transition process.

6.2.4. Impact on protein quality assessment and dietary guidelines

Revised protein estimation methods will also affect protein quality assessments, including the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) and the Digestible Indispensable Amino Acid Score (DIAAS), both of which rely on $N \times 6.25$ (see also section 6.4) (Moore et al. 2010; Rutherfurd et al. 2015). Adjusting protein values in FCDBs may necessitate recalibrating these measures to ensure they reflect the true biological value of dietary proteins. Food-based dietary guidelines (for instance in Germany) and other nutritional recommendations would also need revision to align with the updated data, as they are based on FCDB protein data (Max Rubner-Institut 2014; Schäfer et al. 2024).

6.3. Quantifying the implications of revised protein calculations

To illustrate the impact of a revised protein definition, we compared protein estimates calculated using the standard $N \times 6.25$ method with those derived from amino acid residues and updated, food-specific NCFs (k_p from Mariotti, Tomé, and Mirand (2008)) (see Table 4).

The results show that crude protein values overestimate actual protein content across various food groups, particularly in animal-based products such as meat and fish, where the discrepancy can reach up to 26%. Plant-based foods like legumes and cereals also show notable overestimations, ranging from 10% to over 20%, depending on the calculation method and specific matrix. Although NPN is more prevalent in plant-based foods, the absolute protein overestimation of crude protein values is also high in animal-based products, likely due to differences in protein-nitrogen content. However, NPN significantly influences protein quality assessment, particularly in legumes and cereals, as it contributes to total nitrogen content (see Supplementary Table S1).

These calculations clearly demonstrate that protein values derived using the universal NCF of 6.25 in FCDBs are systematically overestimated. This effect is most striking when considering true protein content, where overestimation reached 10–26% depending on the food group. Using more specific NCFs proposed by Mariotti, Tomé, and Mirand (2008), reduces the discrepancy (typically to 7–14%), it remains substantial.

The observed discrepancies across food matrices can primarily be attributed to two factors, which are complementary to the reasons why the universal factor of 6.25 is flawed (see section 1):

Differences in amino acid composition

The nitrogen content per gram of protein depends on the amino acid profile. Proteins rich in nitrogen-rich amino acids (e.g., arginine, histidine) have a higher nitrogen content, while proteins rich in amino acids with lower nitrogen (e.g., phenylalanine, leucine) have a lower nitrogen content. Fish and cereal proteins frequently exhibit elevated levels of glutamic acid and aspartic acid (Feng et al. 2012; Pferdmenges et al. 2025), which are measured analytically as the combined content of their acid and amide forms. Although the presence of nitrogen-rich amidic forms such as glutamine and asparagine is not directly quantified, their contribution is assumed based on these combined measurements and may explain the overestimation of true protein content when using a fixed factor of 6.25.

Contribution of NPN

NPN compounds – such as nucleic acids, amines, amides, and other small nitrogen-containing molecules – contribute to the total nitrogen content but do not belong to the actual protein fraction. It is well established that fish tissues contain significant amounts of NPN, including trimethylamine oxide (TMAO), creatine/creatinine, and imidazole dipeptides

Table 4. Comparison of current and revised protein estimates (given as g/100g fresh weight) for selected foods.

Food group	Food item	Crude protein content (N x 6.25) [g/100g]	True protein content (sum of amino acid residues)[1]		Protein content from nitrogen with updated Mariotti factors (N x k_p)[2]		N and amino acid contents from reference
			Value [g/100g]	Overestimation of Nx6.25	Value [g/100g] (NCF)	Overestimation of Nx6.25	
Meat	Cattle muscle meat	22.52	19.18*	17.4%	19.73 (5.48)	14.1%	Vopálenský et al. (2017)
Fish	Fish and fish products	16.24	12.86	26.3%	14.50 (5.58)	12.0%	Salo-Väänänen and Koivistoinen (1996)
Dairy	Emmental cheese	28.41	26.54	7.0%	26.59 (5.85)	6.8%	Sieber (2012)
Eggs	Egg, chicken, whole, raw	12.63	11.21	12.7%	11.47 (5.68)	10.1%	Roe et al. (2013)
Cereals	Spelt wholemeal flour	12.76	10.35	23.3%	11.21 (5.49)	13.8%	Pferdmenges et al. (2025)
Legumes	Soy drink	3.23	2.93	10.2%	2.84 (5.50)	13.7%	Stöckl et al. (2024)

*Only values for 16 amino acids available; [1] FAO and WHO (2019) [2] Mariotti, Tomé, and Mirand (2008).

(Van Waarde 1988). Similarly, cereals also contain NPN, although to a lesser extent.

In both food groups – fish and cereals – the combined effect of lower true protein-to-nitrogen ratios and higher NPN fractions explains why the conventional 6.25 can lead to protein overestimation of 26.3% and 23.3%, respectively.

In conclusion, while these calculations highlight the significant extent of protein overestimation using the traditional approach, the development and application of high-quality, food-specific NCFs is essential to accurately reflect protein content according to a revised definition in the future.

6.4. Scope limitation

While the primary objective of this discussion is to refine the definition and quantification of protein in FCDBs, it is essential to acknowledge related considerations that fall outside the scope of this paper.

Firstly, protein content alone does not determine the nutritional value, as it does not account for protein quality, digestibility, or amino acid composition. Protein quality assessments such as PDCAAS and DIAAS evaluate the supply of IAAs and nitrogen needed to meet human requirements. For instance, the FAO/WHO/UNU report estimates an Estimated Average Requirement for protein of 0.66 g/kg/day, with 0.18 g/kg/day for IAAs and 0.48 g/kg/day for DAAs (FAO, WHO, and UNU 2007). These distinctions emphasize that dietary guidelines should not rely solely on protein content but also incorporate digestibility and amino acid profiles.

PDCAAS and DIAAS are widely used to assess protein quality. PDCAAS, introduced by FAO/WHO in the late 1980s, determines protein quality based on the lowest IAA score multiplied by true fecal protein digestibility (FAO and WHO 1991; Schaafsma 2000). However, it accounts only for amino acid replacement rather than optimal metabolism (Schaafsma 2012). DIAAS evaluates ileal amino acid digestibility using human or animal models, comparing digestible IAAs in the test protein to a reference protein (Rutherfurd et al. 2015). Both PDCAAS and DIAAS rely on protein content calculations based on total nitrogen (N x 6.25), which can introduce inaccuracies due to food-specific nitrogen-to-protein variability (Moore et al. 2010; Rutherfurd et al. 2015). Therefore, accurate dietary protein assessment requires quantification of both total protein and amino acid availability, as emphasized by the FAO recommendation to report individual amino acid values in food tables (FAO 2011). While current FCDBs do not include values on bioavailability, future developments may benefit from integrating separate indicators of bioavailability alongside analytical data, particularly for nutrients such as protein where protein digestibility and quality are of nutritional importance.

Furthermore, protein quantification plays a critical role in food safety, quality assurance, and fraud prevention. The economic value of proteins makes them a target for adulteration, as seen in 2007–2008 in China, where melamine was used to falsely boost protein concentrations in infant formulae and pet foods (Sharma and Paradakar 2010). This highlights the limitations of nitrogen-based protein quantification methods, which lack selectivity and fail to analytically exclude NPN. At the same time, it underscores the need for selective, accurate protein determination methods capable of differentiating true protein from nitrogenous adulterants (Moore et al. 2010).

Additionally, it is crucial to recognize that the definition of protein as a nutrient does not always align with the definition of protein as an ingredient. Protein isolates, valued for their high protein content, often contain residual moisture, fat, ash, carbohydrates, vitamins, and other compounds (Holt et al. 1999; Kudre, Benjakul, and Kishimura 2013; Sánchez-Vioque et al. 1999; Etzbach et al. 2024). On the other hand, free amino acids might not be included in protein isolates, leading to differences in analytical methods. Therefore, procedures for isolating protein fractions differ from those used to determine protein content based on amino acid composition, complicating standardization across regulatory and industrial settings.

7. Conclusion

Revisiting the understanding of protein in FCDBs – including its definition, analytical determination, and calculation methods – offers considerable potential for improving the accuracy and consistency of protein data. Bridging theoretical definitions with practical implementation can help mitigate systematic overestimations, support the application of protein analysis across diverse food matrices, and provide more robust data for use in nutrition science, regulatory

affairs, industry, and public health. To support this objective, several key actions are recommended:

First, protein quantification should prioritize amino acid-based determination as the analytical gold standard, given its ability to provide the most precise reflection of true protein content.

Secondly, the use of updated nitrogen-to-protein conversion factors (NCFs), calculated in line with the methodological framework outlined in this manuscript, is strongly advised. A deeper evaluation of the practical impact of the revised definition will become possible once an updated and comprehensive set of NCFs is established – an effort currently underway. This will form the basis for further analytical and conceptual developments, to be shared in future publications.

In addition, promoting greater harmonization of protein calculation practices across institutions and countries will be essential. In parallel and to support both regulatory compliance and scientific advancement, it is recommended that FCDBs include multiple protein values derived from distinct calculation methods. Specifically, crude protein values (based on total nitrogen × 6.25) should still remain available to meet legal and labeling requirements, while a newly defined protein component – based on amino acid residues or improved NCFs – should be introduced. This dual reporting structure would enhance transparency, allow users to choose values suitable for their specific applications, and align with existing database practices for other nutrients, such as carbohydrates.

Finally, translating scientific advancements into actionable guidance will require sustained communication with key stakeholders, including policy makers, database compilers, regulatory authorities, and the food industry. While this review does not aim to resolve those practical challenges, it offers a foundational framework for the upcoming steps toward more precise and harmonized protein data in FCDBs.

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Author contributions

Larissa E. Pferdmenges: Writing - Original Draft, Conceptualization, Methodology; Paolo C. Colombani: Writing - Review & Editing; Monica Hauger Carlsen: Writing - Review & Editing; Anne-Maria Pajari: Writing - Review & Editing; Anders Poulsen: Writing - Review & Editing; M. Graça Dias: Writing - Review & Editing; Anders Møller: Writing - Review & Editing; Silvia Lisciani: Writing - Review & Editing; Matthias Wüst: Writing - Review & Editing; Stefan Storcksdieck genannt Bonsmann: Writing - Review & Editing, Supervision; Ute Schweiggert-Weisz: Writing - Original Draft, Writing - Review & Editing, Supervision. All authors approved the final version of this manuscript and agree to be accountable for all aspects of the work.

Disclosure statement

Paolo C. Colombani is employee of Consulting Colombani GmbH and in this position consults and receives fees from governmental and non-governmental institutions working in the field of nutrition and health, as well as from the food industry and food associations. He is co-founder, editor, and writer of the competence center "Notabene Nutrition", and serves as an expert/science writer for different media in Switzerland.

Use of generative AI

In the preparation of this manuscript, generative AI tools such as ChatGPT 4o and DeepL (Write) were used in order to improve the understanding and readability of the written text.

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References

Agência Nacional de Vigilância Sanitária. 2003. Resolução RDC n° 360, de 23 de dezembro de 2003. In *Diário Oficial da União*. Brasília: Ministério da Saúde.

Anderhuber, N., P. Fladischer, M. Gruber-Khadjawi, J. Mairhofer, G. Striedner, and B. Wiltschi. 2016. High-level biosynthesis of norleucine in E. coli for the economic labeling of proteins. *Journal of Biotechnology* 235:100–11. doi:10.1016/j.jbiotec.2016.04.033.

Biancarosa, I., M. Espe, C. G. Bruckner, S. Heesch, N. Liland, R. Waagbø, B. Torstensen, and E. J. Lock. 2017. Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. *Journal of Applied Phycology* 29 (2):1001–9. doi:10.1007/s10811-016-0984-3.

Boulos, S., A. Tännler, and L. Nyström. 2020. Nitrogen-to-protein conversion factors for edible insects on the Swiss Market: *T. molitor*, *A. domesticus*, and *L. migratoria*. *Frontiers in Nutrition* 7:89. doi:10.3389/fnut.2020.00089.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72 (1-2):248–54. doi:10.1016/0003-2697(76)90527-3.

Brugère, J.-F., J. F. Atkins, P. W. O'Toole, and G. Borrel. 2018. Pyrrolysine in archaea: A 22nd amino acid encoded through a genetic code expansion. *Emerging Topics in Life Sciences* 2 (4):607–18. doi:10.1042/ETLS20180094.

Chang, S. K. C., and Yan Zhang. 2017. Protein analysis. In *Food Analysis*, ed. S. Nielsen, 315-331. Switzerland: Springer International Publishing

Churchward-Venne, T. A., C. H. Murphy, T. M. Longland, and S. M. Phillips. 2013. Role of protein and amino acids in promoting lean mass accretion with resistance exercise and attenuating lean mass loss during energy deficit in humans. *Amino Acids* 45 (2):231–40. doi:10.1007/s00726-013-1506-0.

Code of Federal Regulations. 2024. *Code of Federal Regulations 21 CFR 101.9(c)(7) on food labeling*. United States of America: Food and Drug Administration, Department of Health and Human Services

Dumas, J.-B. A. 1831. Procédés de l'analyse organique. *Annales De Chimie Et De Physique* 47:198–205.

Durazzo, A., S. Astley, M. Kapsokefalou, H. S. Costa, A. Mantur-Vierendeel, L. Pijls, L. Bucchini, M. Glibetić, K. Presser, and P. Finglas. 2022. Food composition data and tools online and their use in research and policy: EuroFIR AISBL contribution in 2022. *Nutrients* 14 (22):4788. doi:10.3390/nu14224788.

EFSA Panel on Dietetic Products, Nutrition, and Allergies. 2012. Scientific opinion on dietary reference values for protein. *EFSA Journal* 10 (2):2557. doi:10.2903/j.efsa.2012.2557.

Estrella, D. A. R. 2008. *Amino acid composition and nitrogen to protein conversion factors for three legumes and two pseudo-cereals*. Quito: USFQ.

Etzbach, L., S. Gola, F. Küllmer, I.-H. Acir, D. Wohlt, L. M. Ignatzy, S. Bader-Mittermaier, and U. Schweiggert-Weisz. 2024. Opportunities and challenges of plant proteins as functional ingredients for food

production. *Proceedings of the National Academy of Sciences of the United States of America* 121 (50):e2319019121. doi:10.1073/pnas.2319019121.

Evers, J., D. Barbano, I. Alper, M. Cameron, B. Farhang, M. Gips, H. Frister, et al. 2016. Evaluation of nitrogen conversion factors for dairy and soy.

FAO. 2003. Food energy – methods of analysis and conversion factors. In *FAO food and nutrition paper*. Rome: FAO Food and Nutrition.

FAO. 2011. Dietary protein quality evaluation in human nutrition: Report of an FAO Expert Consultation. In *FAO food and nutrition paper*, 92:1–66.

FAO. 2013. Dietary protein quality evaluation in human nutrition. Report of an FAO expert consultation, 31 March-2 April, 2011, Auckland, New Zealand. In *FAO food and nutrition paper*. Rome: FAO Food and Nutrition.

FAO and WHO. 1985. Guidelines on nutrition labelling CAC/GL 2–1985.

FAO and WHO. 1991. "Protein quality evaluation. Joint FAO/WHO. In *FAO Food and Nutrition Paper* 51:1–66.

FAO and WHO. 2019. *Nitrogen and protein content measurement and nitrogen to protein conversion factors for dairy and soy protein-based foods: A systematic review and modelling analysis*. Geneva: World Health Organization.

FAO, WHO, and UNU. 2007. Protein and amino acid requirements in human nutrition: Report of a joint FAO/WHO/UNU expert consultation.

Feng, Y. W., L. Z. Ma, Y. C. Du, S. H. Fan, and R. T. Dai. 2012. Chemical composition analysis of three commercially important fish species (Sardine, Anchovy and Mackerel). *Advanced Materials Research* 554-556:900–4. doi:10.4028/www.scientific.net/AMR.554-556.900.

Ferrando, A. A., R. R. Wolfe, K. R. Hirsch, D. D. Church, S. A. Kviatkovsky, M. D. Roberts, J. R. Stout, D. E. Gonzalez, R. J. Sowinski, R. B. Kreider, et al. 2023. International Society of Sports Nutrition Position Stand: Effects of essential amino acid supplementation on exercise and performance. *Journal of the International Society of Sports Nutrition* 20 (1):2263409. doi:10.1080/15502783.2023.2263409.

Friedman, M. 1999. Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins. *Journal of Agricultural and Food Chemistry* 47 (4):1295–319. doi:10.1021/jf981000+.

Fujihara, S., A. Kasuga, and Y. Aoyagi. 2001. Nitrogen-to-protein conversion factors for common vegetables in Japan. *Journal of Food Science* 66 (3):412–5. doi:10.1111/j.1365-2621.2001.tb16119.x.

Fujihara, S., A. Kasuga, Y. Aoyagi, and T. Sugahara. 1995. Nitrogen-to-protein conversion factors for some common edible mushrooms. *Journal of Food Science* 60 (5):1045–7. doi:10.1111/j.1365-2621.1995.tb06289.x.

Fujihara, S., H. Sasaki, Y. Aoyagi, and T. Sugahara. 2008. Nitrogen-to-protein conversion factors for some cereal products in Japan. *Journal of Food Science* 73 (3):C204–C209. doi:10.1111/j.1750-3841.2008.00665.x.

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

Gaßmann, B. 2006. Aminosäuren und Proteine, Teil 2: Proteine. *Ernährungs-Umschau* 53 (5):176–81.

Greenfield, H., and D. A. T. Southgate. 2003. *food composition data: Production, management and use*. 2nd ed. Rome: Food and Agriculture Organization of the United Nations.

Hartree, E. F. 1972. Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry* 48 (2):422–7. doi:10.1016/0003-2697(72)90094-2.

Hayes, M. 2020. Measuring protein content in food: An overview of methods. *Foods (Basel, Switzerland)* 9 (10):1340. doi:10.3390/foods9101340.

Heinrich, P. C., M. Müller, L. Graeve, and H.-G. Koch. 2022. *Löffler/Petrides Biochemie und Pathobiochemie*. Berlin: Springer.

Holt, C., D. McPhail, I. Nevison, T. Nylander, J. Otte, R. H. Ipsen, R. Bauer, L. Ōgendal, K. Olieman, K. G. deKruif, et al. 1999. Apparent chemical composition of nine commercial or semi-commercial whey protein concentrates, isolates and fractions. *International Journal of Food Science and Technology* 34 (5-6):543–56. doi:10.1046/j.1365-2621.1999.00323.x.

Holzmüller, W., and U. Kulozik. 2016. Protein quantification by means of a stain-free SDS-PAGE technology without the need for analytical standards: Verification and validation of the method. *Journal of Food Composition and Analysis* 48:128–34. doi:10.1016/j.jfca.2016.03.003.

Hu, S., W. He, and G. Wu. 2022. Hydroxyproline in animal metabolism, nutrition, and cell signaling. *Amino Acids* 54 (4):513–28. doi:10.1007/s00726-021-03056-x.

Imafidon, G. I., and F. W. Sosulski. 1990. Nonprotein nitrogen contents of animal and plant foods. *Journal of Agricultural and Food Chemistry* 38 (1):114–8. doi:10.1021/jf00091a023.

Ingle, P. D., R. Christian, P. Purohit, V. Zarraga, E. Handley, K. Freel, and S. Abdo. 2016. Determination of protein content by NIR spectroscopy in protein powder mix products. *Journal of AOAC International* 99 (2):360–3. doi:10.5740/jaoacint.15-0115.

Johansson, L., G. Gafvelin, and E. S. J. Arnér. 2005. Selenocysteine in proteins-properties and biotechnological use. *Biochimica Et Biophysica Acta* 1726 (1):1–13. doi:10.1016/j.bbagen.2005.05.010.

Jones, D. B. 1941. Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. *United States Department of Agriculture, Circular Series* 183:1–21.

Jung, S., D. A. Rickert, N. A. Deak, E. D. Aldin, J. Recknor, L. A. Johnson, and P. A. Murphy. 2003. Comparison of kjeldahl and dumas methods for determining protein contents of soybean products. *Journal of the American Oil Chemists' Society* 80 (12):1169–73. doi:10.1007/s11746-003-0837-3.

Kamizake, N. K. K., M. M. Gonçalves, C. T. B. V. Zaia, and D. A. M. Zaia. 2003. Determination of total proteins in cow milk powder samples: A comparative study between the Kjeldahl method and spectrophotometric methods. *Journal of Food Composition and Analysis* 16 (4):507–16. doi:10.1016/S0889-1575(03)00004-8.

Kays, S. E., F. E. Barton, and W. R. Windham. 2000. Predicting protein content by near infrared reflectance spectroscopy in diverse cereal food products. *Journal of near Infrared Spectroscopy* 8 (1):35–43. doi:10.1255/jnirs.262.

Kjeldahl, J. 1883. Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. *Fresenius' Zeitschrift Für Analytische Chemie* 22 (1):366–82. doi:10.1007/BF01338151.

Koletzko, B., and R. Shamir. 2006. Standards for infant formula milk. *BMJ (Clinical Research Ed.)* 332 (7542):621–2. doi:10.1136/bmj.332.7542.621.

Krul, E. S. 2019. Calculation of nitrogen-to-protein conversion factors: A review with a focus on soy protein. *Journal of the American Oil Chemists' Society* 96 (4):339–64. doi:10.1002/aocs.12196.

Kudre, T. G., S. Benjakul, and H. Kishimura. 2013. Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *Journal of the Science of Food and Agriculture* 93 (10):2429–36. doi:10.1002/jsfa.6052.

Lelais, G., and D. Seebach. 2004. amino acids—syntheses, occurrence in natural products, and components of β-peptides1,2. *Peptide Science* 76 (3):206–43. doi:10.1002/bip.20088.

Lopez, M. J., and S. S. Mohiuddin. 2024. Biochemisry, essential amino acids. StatPearls Publishing, Accessed February 4, 2024. https://www.ncbi.nlm.nih.gov/books/NBK557845/.

Lowry, O., H. Nira, J. Rosebrough, A. L. Farr, Rose, and J. Randall. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193 (1):265–75. doi:10.1016/S0021-9258(19)52451-6.

Lynch, J. M., and D. M. Barbano. 1999. Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. *Journal of AOAC International* 82 (6):1389–98. doi:10.1093/jaoac/82.6.1389.

Maclean, W., J. Harnly, J. Chen, S. Chevassus-Agnes, G. Gilani, G. Livesey, and P. Warwick. 2003. Food energy–Methods of analysis and conversion factors. Paper presented at the FAO of the United Nations Technical workshop report.

Mæhre, H., L. Dalheim, G. Edvinsen, E. Elvevoll, and I.-J. Jensen. 2018. Protein determination - Method matters. *Foods* 7 (1):5. doi:10.3390/foods7010005.

Mariotti, F., D. Tomé, and P. P. Mirand. 2008. Converting nitrogen into protein—Beyond 6.25 and Jones' factors. *Critical Reviews in Food Science and Nutrition* 48 (2):177–84. doi:10.1080/10408390701279749.

Maubois, J. L., and D. Lorient. 2016. Dairy proteins and soy proteins in infant foods nitrogen-to-protein conversion factors. *Dairy Science & Technology* 96 (1):15–25. doi:10.1007/s13594-015-0271-0.

Max Rubner-Institut. 2014. Bundeslebensmittelschlüssel (BLS): Version 3.02 (German Nutrient Database: Version 3.02). MRI. Accessed October 16, 2024. http://www.blsdb.de.

McKerchar, H., J. M. Dyer, J. A. Gerrard, E. Maes, S. Clerens, and R. C. J. Dobson. 2023. Characterizing lysinoalanine crosslinks in food systems: Discovery of a diagnostic ion in model peptides using MALDI mass spectrometry. *Food Chemistry: X* 19:100800. doi:10.1016/j.fochx.2023.100800.

Meade, S. J., E. A. Reid, and J. A. Gerrard. 2005. The impact of processing on the nutritional quality of food proteins. *Journal of AOAC International* 88 (3):904–22. doi:10.1093/jaoac/88.3.904.

Ministry of Health of the PRC. 2021. *National food safety standard - General rules for nutrition labeling of prepackaged food. GB28050-2021*. Beijing: Ministry of Health of the PRC Beijing.

Misra, J. 2001. Variation in nitrogen-to-protein conversion factor for peanut. *Peanut Science* 28 (2):48–51. doi:10.3146/i0095-3679-28-2-2.

Moore, J. C., J. W. Devries, M. Lipp, J. C. Griffiths, and D. R. Abernethy. 2010. Total protein methods and their potential utility to reduce the risk of food protein adulteration. *Comprehensive Reviews in Food Science and Food Safety* 9 (4):330–57. doi:10.1111/j.1541-4337.2010.00114.x.

Morgan, P. T., O. C. Witard, G. Højfeldt, D. D. Church, and L. Breen. 2023. Dietary protein recommendations to support healthy muscle ageing in the 21st century and beyond: Considerations and future directions. *Proceedings of the Nutrition Society*:1–14. doi:10.1017/s0029665123003750.

Morr, C. V. 1982. Recalculated nitrogen conversion factors for several soybean protein products. *Journal of Food Science* 47 (5):1751–2. doi:10.1111/j.1365-2621.1982.tb05032.x.

Mossé, J. 1990. Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. *Journal of Agricultural and Food Chemistry* 38 (1):18–24.

Moughan, P. J. 2016. Protein: Digestion, absorption and metabolism. *Encyclopedia of Food and Health*.Oxford: Academic Press. doi:10.1016/B978-0-12-384947-2.00572-9.

Mulder, G. J. 1838. Over proteine en hare verbindingen en ontledings-producten. *Natuur En Scheikundig Archief* VI:87–162.

Mulder, G. J. 1839. Ueber die Zusammensetzung einiger thierischen Substanzen. *Journal Für Praktische Chemie* 16 (1):129–52. doi:10.1002/prac.18390160137.

Müller, J. 2017. *Dumas or Kjeldahl for reference analysis?* FOSS.

Myllyharju, J. 2005. Intracellular post-translational modifications of collagens. In *Collagen: Primer in structure, processing and assembly*, ed. J. Brinckmann, H. Notbohm and P. K. Müller, 115–47. Berlin: Springer.

Official Journal of the European Union. 2011. Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011.

Otter, D. E. 2012. Standardised methods for amino acid analysis of food. *The British Journal of Nutrition* 108 Suppl 2 (S2):S230–S237. doi:10.1017/S0007114512002486.

Periago, M. J., G. Ros, C. Martínez, and F. Rincón. 1996. Variations of non-protein nitrogen in six Spanish legumes according to the extraction method used. *Food Research International* 29 (5-6):489–94. doi:10.1016/S0963-9969(96)00053-1.

Pferdmenges, L. E., R. Lohmayer, L. Frommherz, L. Brühl, A. Hüsken, E. Mayer-Miebach, A.-K. Meinhardt, U. Ostermeyer, E. Sciurba, H. Zentgraf, et al. 2025. Overview of the nutrient composition of selected milling products from rye, spelt and wheat in Germany. *Journal of Food Composition and Analysis* 140:107275. doi:10.1016/j.jfca.2025.107275.

Ramos-Ruiz, R., F. Martinez, and G. Knauf-Beiter. 2019. The effects of GABA in plants. *Cogent Food & Agriculture* 5 (1):1670553. doi:10.1080/23311932.2019.1670553.

Roe, M., H. Pinchen, S. Church, and P. Finglas. 2013. *Nutrient analysis of eggs*. London: Department of Health.

Rutherfurd, S. M., and B. M. Dunn. 2011. Quantitative amino acid analysis. *Current Protocols in Protein Science* Chapter 3 (1):3.2.1–.2.6. doi:10.1002/0471140864.ps0302s63.

Rutherfurd, S. M., A. C. Fanning, B. J. Miller, and P. J. Moughan. 2015. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats 1, 2, 3. *The Journal of Nutrition* 145 (2):372–9. doi:10.3945/jn.114.195438.

Sáez-Plaza, P., T. Michałowski, M. J. Navas, A. G. Asuero, and S. Wybraniec. 2013. An overview of the Kjeldahl method of nitrogen determination. Part I. Early history, chemistry of the procedure, and titrimetric Finish. *Critical Reviews in Analytical Chemistry* 43 (4):178–223. doi:10.1080/10408347.2012.751786.

Salo-Väänänen, P. P., and P. E. Koivistoinen. 1996. Determination of protein in foods: Comparison of net protein and crude protein (Nx6.25) values. *Food Chemistry* 57 (1):27–31. doi:10.1016/0308-8146(96)00157-4.

San Gabriel, A., and H. Uneyama. 2013. Amino acid sensing in the gastrointestinal tract. *Amino Acids* 45 (3):451–61. doi:10.1007/s00726-012-1371-2.

Sánchez-Vioque, R., A. Clemente, J. Vioque, J. Bautista, and F. Millán. 1999. Protein isolates from chickpea (Cicer arietinum L.): chemical composition, functional properties and protein characterization. *Food Chemistry* 64 (2):237–43. doi:10.1016/S0308-8146(98)00133-2.

Sanvictores, T., and F. Farci. 2022. *Biochemistry, primary protein structure*. Treasure Island, FL: StatPearls Publishing.

Schaafsma, G. 2000. The protein digestibility-corrected amino acid score. *The Journal of Nutrition* 130 (7):1865s–7s. doi:10.1093/jn/130.7.1865S.

Schaafsma, G. 2012. Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. *The British Journal of Nutrition* 108 Suppl 2 (S2):S333–S336. doi:10.1017/s0007114512002541.

Schäfer, A., Carolin, H. Boeing, J. Conrad, Bernhard, W. Für Die, D. G. E. Arbeitsgruppe, and L. Ernährungsempfehlungen. 2024. Wissenschaftliche Grundlagen der lebensmittelbezogenen Ernährungsempfehlungen für Deutschland. Methodik und Ableitungskonzepte. *Ernährungs-Umschau* 71 (3):M158–166. e155–157.

Schweiggert-Weisz, U., L. Etzbach, S. Gola, S. E. Kulling, C. Diekmann, S. Egert, and H. Daniel. 2024. Opinion piece: New plant-based food products between technology and physiology. *Molecular Nutrition & Food Research* 68 (20):e2400376. doi:10.1002/mnfr.202400376.

Sharma, K., and M. Paradakar. 2010. The melamine adulteration scandal. *Food Security* 2 (1):97–107. doi:10.1007/s12571-009-0048-5.

Sieber, R. 2012. Zusammensetzung von Milch und Milchprodukten schweizerischer Herkunft. In *ALP Science* Forschungsanstalt Agroscope Liebefeld-Posieux ALP 538:40.

Simonne, A. H., E. H. Simonne, R. R. Eitenmiller, H. A. Mills, and C. P. Cresman. 1997. Could the Dumas method replace the Kjeldahl digestion for nitrogen and crude protein determinations in foods? *Journal of the Science of Food and Agriculture* 73 (1):39–45. doi:10.1002/(SICI)1097-0010(199701)73:1<39::AID-JSFA717>3.0.CO;2-4.

Söderbaum, H. G. 1916. *Jac. Berzelius Bref*. Uppsala: Kungl. Svenska Vetenskapsakademien (V. Brefväxling mellan Berzelius och G.J.Mulder (1834–1847).

Sosulski, F. W., and G. I. Imafidon. 1990. Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. *Journal of Agricultural and Food Chemistry* 38 (6):1351–6. doi:10.1021/jf00096a011.

Stöckl, M., L. E. Pferdmenges, L. Brühl, R. Greiner, A. Hüsken, R. Krüger, G. Langenkämper, A. Lencioni, A. Müller, M. Schmidt, et al. 2024. Characterization of the nutritional profile of three plant-based drinks. *Journal of Food Composition and Analysis* 135:106553. doi:10.1016/j.jfca.2024.106553.

Thompson, M., L. Owen, K. Wilkinson, R. Wood, and A. Damant. 2002. A comparison of the Kjeldahl and Dumas methods for the determination of protein in foods, using data from a proficiency testing scheme. *The Analyst* 127 (12):1666–8. doi:10.1039/B208973B.

Tkachuk, R. 1969. Nitrogen-to-protein conversion factors for cereals and oilseed meals. *Cereal Chemistry* 46 (4):419–23.

Tomé, D. 2022. Efficiency of free amino acids in supporting muscle protein synthesis. *The Journal of Nutrition* 152 (1):3–4. doi:10.1093/jn/nxab370.

Torten, J., and J. R. Whitaker. 1964. Evaluation of the Biuret and dye-binding methods for protein determination in meats. *Journal of Food Science* 29 (2):168–74. doi:10.1111/j.1365-2621.1964.tb01713.x.

Van Waarde, A. 1988. Biochemistry of non-protein nitrogenous compounds in fish including the use of amino acids for anaerobic energy production. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 91 (2):207–28. doi:10.1016/0305-0491(88)90136-8.

Voet, D., and J. G. Voet. 2010. *Biochemistry.*New Jersey, USA: John Wiley & Sons.

Vopálenský, J., P. Suchý, E. Straková, F. Šimek, M. Macháček, and I. Herzig. 2017. Amino acid levels in muscle tissue of eight meat cattle breeds. *Czech Journal of Animal Science* 62 (8):339–46. doi:10.17221/96/2016-CJAS.

Weijzen, M. E. G., R. J. J. van Gassel, I. W. K. Kouw, J. Trommelen, S. H. M. Gorissen, J. van Kranenburg, J. P. B. Goessens, M. C. G. van de Poll, L. B. Verdijk, and L. J. C. van Loon. 2022. Ingestion of free amino acids compared with an equivalent amount of intact protein results in more rapid amino acid absorption and greater postprandial plasma amino acid availability without affecting muscle protein synthesis rates in young adults in a double-blind randomized trial. *The Journal of Nutrition* 152 (1):59–67. doi:10.1093/jn/nxab305.

WHO, and FAO. 2019. Nitrogen and protein content measurement and nitrogen to protein conversion factors for dairy and soy protein-based foods: A systematic review and modelling analysis.

Wu, G. 2013. *Amino acids: Biochemistry and nutrition*. 1st ed. Boca Raton: CRC Press.

Wu, G., F. W. Bazer, R. C. Burghardt, G. A. Johnson, S. W. Kim, D. A. Knabe, P. Li, X. Li, J. R. McKnight, M. C. Satterfield, et al. 2011. Proline and hydroxyproline metabolism: Implications for animal and human nutrition. *Amino Acids* 40 (4):1053–63. doi:10.1007/s00726-010-0715-z.

Yeoh, H.-H., and V.-D. Truong. 1996. Protein contents, amino acid compositions and nitrogen-to-protein conversion factors for cassava roots. *Journal of the Science of Food and Agriculture* 70 (1):51–4. doi:10.1002/(sici)1097-0010(199601)70:1<51::Aid-jsfa463>3.0.Co;2-w.