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Determination of protein in foods: comparison of net protein and crude protein (N×6.25) values

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The purpose of the present study was: (1) to determine net (true) protein (NP) values as sums of amino acid residues and to compare them with crude protein (PA) values; and (2) to calculate the nitrogen-to-NP conversion factors by means of linear regression analyses between the NP values and nitrogen content. The differences between NP and PA values varied between food groups. The NP values in milk and milk products were, on average, 5.5% smaller than the corresponding PA values. The corresponding figures for other foods were: meat and meat products 15.4%, fish and fish products 20.6%, cereals and bakery products 12.2%, vegetables, fruits and berries 13.9% and miscellaneous processed foods 16.1%. In the present study the nitrogen-to-NP conversion factor for milk and milk products was 5.94, meat and meat products 5.17, fish and fish products 4.94, cereals and bakery products 5.40, vegetables, fruits and berries 5.36 and miscellaneous processed foods 5.51; the general conversion factor for all foods was 5.33. The above respective factors were 5.0, 17.3, 21.0, 13.6, 14.2, 11.8 and 14.7% smaller than the official factor of 6.25. We conclude that the traditional PA values (N×6.25) deviate significantly from the NP values. We recommend that protein definitions and methods of determination should be re-evaluated in the appropriate international organizations and updated in line with current knowledge and analytical capabilities for both scientific and other purposes. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Protein content is one of the most essential nutritional attributes of foods; it is determined by 20 L-amino acids bound in proteins and peptides or occurring in minor amounts in free form. The true quantitative protein value for a food is thus determined by the total amount of the 20 bound and free amino acids (Food and Nutrition Board, 1989), and is referred to in the present paper as the net protein of a food.

Early methods for quantification of proteins in natural materials were based on the observation that some animal proteins contain about 16% nitrogen (e.g. Henneberg, 1865; Rubner, 1885; Atwater & Bryant, 1899; Jones, 1931; Browne, 1944). It was assumed (Henneberg, 1865) that the protein content of a natural product in general could be calculated by multiplying the total nitrogen content by a conversion factor of 6.25. It has long been known (Jones, 1931) that this procedure involves two major misconceptions: (1) in a product, varying amounts of nitrogen are derived from substances other than the 20 bound or free amino acids; and (2) the nitrogen content of proteins may vary significantly between and within products. Product-specific nitrogen-to-protein conversion factors have been determined especially for plant materials (e.g. Jones, 1931; Heatcote, 1950; Tkachuk, 1969; Sosulski & Holt, 1980; Mossé, 1990; Yeoh & Wee, 1994), feeds (Boisen *et al.*, 1987), animal and plant products (Sosulski & Imafidon, 1990) and processed foods and diets (Lebet *et al.*, 1994).

Since the traditional procedure for determining protein in foods (N×6.25) is still officially endorsed in Finland (Ministry of Trade and Industry Regulations, 1993), in the EU (Council Directive, 1990) and in the USA (Code of Federal Regulations, 1993) for food labelling and is broadly used for other purposes, we considered it desirable to study a wide variety of foods and believe it to be the most comprehensive such study thus far conducted. We compared the net (true) protein (NP) values, i.e. the sum of amino acid residues, with the crude protein (PA) values (N×6.25) in different food groups and also calculated the nitrogen-to-NP conversion factors by means of linear regression analyses between NP values and the nitrogen contents for six food groups and a general factor for all foods.

MATERIALS AND METHODS

In our study the amino acid compositions and nitrogen content of 148 different kinds of food were determined. The foods were grouped as follows.

1. Milk and milk products, including human milk, eggs and infant formulas (28 items).
2. Meats of various animals and meat products, including edible offals (32 items).
3. Fish and fish products, including shrimp and roe (28 items).
4. Cereals and bakery products (19 items).
5. Vegetables, fruits, and berries and their products, including root crop (24 items).
6. Miscellaneous processed foods, mainly ready-to-eat products from mixed raw materials (17 items).

Samples of the most widely selling brands were, with some exceptions, purchased in 10 stores of the four major retail (>90% of total sales) food chains in the Helsinki area. All 10 subsamples of each food item were mixed, minced or cut in small pieces and pooled; only the edible part of each sample was used. The pooled samples were considered adequate for the purposes of our study, and were freeze-dried, ground, vacuum-packed and stored at −20°C until analysis.

The nitrogen contents of the samples were determined by the standard Kjeldahl procedure of the AOAC (1990), using 12 ml of concentrated H_2SO_4 (95–97%, p.a., Merck) and K_2SO_4–$CuSO_4$ (Special Kjeltabs C 3.5, Tecator, Sweden) as catalyst. The temperature of the hydrolysis was 410°C and the time 1.5 h (Tecator Digestion System 20, 1015 Digestor, Tecator, Sweden). The nitrogen content was determined as a boric acid application with the Kjeltec Auto 1030 Analyser (Tecator, Sweden).

The amino acid contents of the pooled samples, except that of tryptophan, were analysed with an amino acid analyser, the tryptophan was measured separately as a norharman derivative with a fluorometer. For determinations with the amino acid analyser, the freeze-dried, homogenized samples containing 0.04–0.07 g protein were acid-hydrolysed with 40 ml of 6 N HCl (diluted 1:1 from 37% HCl, p.a., Merck) at 110°C for 24 h under nitrogen (Gehrke *et al.*, 1985, 1987; AOAC, 1990). The cysteine was measured as cysteic acid, and methionine as methionine sulphoxides and methionine sulphone after performic acid (1:9 mixture of 30% H_2O_2; p.a. and 98–100% HCOOH, p.a.; Merck) oxidation and 6 N HCl hydrolysis under reflux according to Mason *et al.* (1980*a*,*b*), Andersen *et al.* (1984) and AOAC (1990). An LKB Alpha 4150 amino acid analyser (LKB, Biochrom, UK) was used in applying the accelerated procedure of Condon (1986) and Williams (1988). Twenty protein amino acids were determined. The amino acid analyser detected 17 amino acids, as during acid hydrolysis asparagine was converted into aspartic acid and glutamine into glutamic acid. The pooled samples were analysed in duplicate. The tryptohan was determined with a Perkin-Elmer 3000 fluorometer (Perkin Elmer Ltd, UK) on a $Ba(OH)_2$ hydrolysate (Steinhart, 1979; Piombo & Lozano, 1980; Nielsen & Hurrell, 1985; Scheuerman & Eckstein, 1986) as a norharman derivative (Gaitonde *et al.*, 1979; Sachse, 1981). The tryptophan analysis of pooled samples was done in triplicate.

The digestion step of the Kjeldahl method is easy to control by adjusting the temperature, time and the amount of reagents used. Validation of the accuracy and precision of the Kjeldahl method was tested by daily determining nitrogen levels (distillation and ammonia measurement) of the 5% NH_4Cl ($n=20$). The accuracy of the nitrogen determination varied between 97.2 and 99.1% (mean 98.2%) and the precision (CV%) was 0.5%. These values were in the same range as those of Fisher & Gurnsey (1987), although CV% was significantly lower than that of Watkins *et al.* (1987).

For validation of the analytical methods, the accuracy and precision of the amino acid analyses were tested by using hydrolysates of ANRC reference protein (NBCo Biochemicals, USA) or β-lactoglobulin (B, Sigma, L-8005, USA). The accuracy of the amino acid (except tryptophan) analyses varied from 86.8% for serine to 110% for arginine but was mainly in the range 90.5–105.3% ($n=20$). The accuracy of tryptophan analyses was 99.2% ($n=48$). These values were in accordance with those of Sachse (1981), MacDonald *et al.* (1985) and Lebet *et al.* (1994). The CV% of amino acid analyses (except tryptophan) varied between 3.0 and 9.8% ($n=20$), and was smaller than that of Williams (1988) and slightly higher than that of Phillips (1983). The CV% of tryptophan analyses was 4.4% ($n=48$) and was in the same range as that of Sachse (1981). The net protein content (i.e. the sum of amino acid residues) of reference proteins compared to their crude protein values varied between 93.7 and 99.2%. The average was 95.2%, which was the same as that of Tkachuk (1969). The amino acid values were not corrected since there are no reliable correction factors (Rowan *et al.*, 1992), although minor losses may occur because of the single hydrolysis time and carbohydrates.

Table 1. New nitrogen-to-net protein conversion factors for food groups and all foods

Foods	Factor ± 2× standard error	Variance	Standard deviation	Deviation from 6.25	
				units	%
1. Milk and milk products	5.94 ± 0.18	1.37	±1.17	−0.31	−5.0
2. Meat and meat products	5.17 ± 0.18	1.86	±1.36	−1.08	−17.3
3. Fish and fish products	4.94 ± 0.12	0.74	±0.86	−1.31	−21.0
4. Cereals and bakery products	5.40 ± 0.30	1.0	±1.0	−0.85	−13.6
5. Vegetables, fruits and berries	5.36 ± 0.30	0.03	±0.19	−0.89	−14.2
6. Miscellaneous processed foods	5.51 ± 0.30	0.64	±0.80	−0.74	−11.8
All foods	5.33 ± 0.10	1.65	±1.28	−0.92	−14.7

Calculation of the conversion factors

In our study we calculated the specific conversion factors for each food group and a general conversion factor for all foods by means of linear regression analyses between the NP and the Kjeldahl-nitrogen values. The confidence limits of each factor were calculated (2×standard error of the linear regression analyses). The regression analysis models were forced through the origin, since the *P* values of the linear function constants were not significant.

RESULTS AND DISCUSSION

For comparison of the traditional PA values (N×6.25) with the NP values of the present study, new nitrogen-to-NP conversion factors for the six food groups and a general factor for all foods were calculated and are reported in Table 1. Regression analysis of the general factor of 5.33 shows good correlation between the nitrogen content and the NP values (Fig. 1). The conversion factors found were substantionally 5–20% smaller than the traditional factor of 6.25: the general factor about 15%. These figures indicate that a significant amount of nitrogen was derived from compounds which do not occur in amino acid structures. Fish and fish products and meat and meat products contained these compounds in particularly large amounts, probably due to both natural composition and degradation of proteins, while deviation of milk products was the smallest.

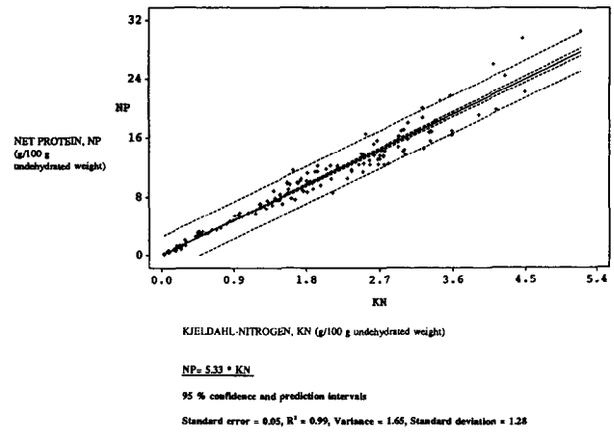

Fig. 1. Linear regression analysis between net protein and Kjeldahl-nitrogen.

The protein values obtained by various methods are given in Table 2 for each food group:

1. (NP) as the sum of amino acid residues;
2. (PA) as N×6.25; and
3. calculated net protein value (PB) as N×5.33.

The NP values in milk and milk products averaged 5.5%, meat and meat products 15.4%, fish and fish products 20.6%, cereals and bakery products 12.2%, vegetables, fruits and berries 13.9% and miscellaneous processed foods 16.1% smaller than the corresponding PA values. These figures are in the same range as those of Lebet *et al.* (1994).

Table 2. Average protein values of food groups obtained by different procedures and differences of values (NP, net protein as sum of amino acid residues; PA, crude protein as N×6.25; PB, net protein calculated as N×5.33)

Food item	NP	PA	PA-NP		PB	PB-NP	
	g/100 g[1]	N×6.25	g/100 g[1]	%	N×5.33	g/100 g[1]	%
Milk and milk products							
Mean ($n=28$)	11.13	11.72	0.59	5.5	10.00	−1.13	−10.8
Range	0.84–30.40	1.01–32.44	−1.56 to 5.51	−9.8 to 26.9	0.86–27.66	−5.67 to 1.90	−28.7 to 14.5
Standard deviation	±9.49	±9.91	±1.27	±9.1	±8.45	±1.52	±10.7
Meat and meat products							
Mean ($n=32$)	13.49	16.09	2.60	15.4	13.78	0.29	1.2
Range	7.59–21.01	8.90–25.88	0.17–5.60	1.5–27.2	7.59–22.07	−2.64 to 2.78	−15.6 to 16.1
Standard deviation	±3.57	±4.44	±1.69	±8.1	±3.84	±1.40	±9.7
Fish and fish products							
Mean ($n=28$)	12.86	16.24	3.38	20.6	13.85	0.98	6.9
Range	4.74–22.14	5.56–28.04	0.60–6.05	5.0–29.3	4.74–23.92	−1.16 to 2.31	−11.4 to 17.2
Standard deviation	±3.46	±4.33	±1.31	±5.5	±3.70	±0.93	±9.6
Cereal and bakery products							
Mean ($n=19$)	7.86	9.01	1.14	12.2	7.68	−0.18	−3.0
Range	4.55–12.15	5.28–13.33	−0.07 to 4.81	−0.8 to 36.1	4.50–11.37	−1.84 to 2.85	−18.2 to 25.1
Standard deviation	±2.01	±2.31	±1.10	±8.9	±1.67	±0.98	±10.5
Vegetables, fruits and berries							
Mean ($n=24$)	1.08	1.27	0.19	13.9	1.04	−0.00	−0.9
Range	0.16–4.24	0.21–5.09	−0.28 to 0.53	−19.6 to 46.5	0.18–4.34	−0.55 to 0.37	−41.9 to 37.8
Standard deviation	±0.88	±1.01	±0.24	±16.5	±0.87	±0.19	±19.6
Miscellaneous processed foods							
Mean ($n=17$)	6.47	7.38	0.92	16.1	6.29	−0.17	1.5
Range	0.10–12.16	0.17–13.70	−1.33 to 2.31	−13.0 to 46.7	0.14–11.68	−2.84 to 0.81	−32.4 to 37.7
Standard deviation	±4.09	±4.46	±0.91	±13.4	±3.80	±0.82	±15.4

[1]Undehydrated weight.

When our general conversion factor of 5.33 was applied to various food groups, it generally resulted in values (PB) very close to the NP values expressed as the sum of amino acid residues, except in the milk and milk products group in which the PB values underestimated the NP values by 10%. The food group-specific conversion factors, as expected, resulted in values matching well with the NP values within the specific groups.

CONCLUSIONS

We conclude that the NP values determined as sums of amino acid residues are, depending on the type of food group, up to 20% smaller than the PA values. In consequence, we highly recommend that the protein values of foods should be based on the direct quantitative analysis of amino acids or indirectly on the conversion factors (nitrogen-to-NP) with the prerequisite that an adequate uniformity of the true protein/total nitrogen ratio in a food product is guaranteed. The present official conversion factor of 6.25 overestimates the true protein content of foods and other biological materials to the extent that its acceptability as a method for determining protein content, e.g. for food labelling, commercial product declarations or food databanks is questionable. We recommend that international measures used by appropriate organizations should be re-evaluated with regard to the definitions and methods of determination of proteins in food, feed, raw materials and processed products for scientific and other purposes.

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