A perspective on the validity of using Jones factors to calculate protein content

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Introduction

Nitrogen to Protein Conversion Factors (NPCFs), also known as Jones factors, enable the estimation of the protein content within food samples by assessing the nitrogen quantity in the food. This relies on two underlying assumptions: firstly, that the majority of nitrogen is linked to amino acids, and secondly, that a significant portion of the amino acids in foods is connected to protein. The accuracy of this estimation depends on the specific value of the conversion factor used. Typically, a value of 6.25 is utilized to determine protein content in the majority of foods. This value is based on two assumptions: firstly, proteins contain approximately 16% nitrogen by weight (meaning 16% of the total protein mass is composed of nitrogen), and secondly, that all nitrogen present in food originates from protein. Nonetheless, applying the identical conversion factor across all sources of protein can pose limitations.

In this study, the amino acid content of high-protein foodstuffs (meat, dairy and protein supplements) were assessed and compared in two ways. First, using the respective Jones factors applied to the total nitrogen determined and secondly by using the sum of the amino acid residues (1).

Methodology

Nitrogen and the respective amino acid data of animal protein source foods and sports supplements in South Africa were extracted from published literature and from unpublished reports, where available.

Nitrogen analysis and protein calculation using Jones factors

The nitrogen of the samples was determined by either the Kjeldahl method (dairy samples) or Dumas method (meat and sport supplements). The nitrogen content was then used to calculate crude protein using the respective Jones conversion factors of 6.38 (dairy products and dairy based sport supplements), 5.71 (soy protein based supplements) and 6.25 for all the other samples.

Determination of amino acids and calculation of protein content

The amino acid profile was determined by using high-performance liquid chromatography (HPLC) equipped with an AMinoTAg column and florescence detection. The determination was carried out during three separate hydrolysis. The first hydrolysis analysed arginine, hydroxyproline, serine, aspartic acid, glutamic acid, threonine, glycine, alanine, tyrosine, proline, methionine, valine, phenylalanine, isoleucine, leucine, histidine and lysine. The samples were weighed and hydrolysed with 6 N hydrochloric acid. An internal standard was added to the hydrolysate and filtered. A portion of the hydrolysate was dried under nitrogen-flow. The hydrolysate was derivatised with FMOC reagent of 9-fluorenylmethyl chloroformate and the amino acid content was determined by HPLC with an eluent of a tertiary gradient of pH, methanol and acetonitrile.

The second hydrolysis determined cysteine and followed an identical approach as described above with the exception that prior to hydrolysis cysteine was oxidised to cystic acid with a peroxide formic acid solution. The third hydrolysis determined tryptophan. Samples were hydrolysed enzymatically using protease. The hydrolysis was filtered and tryptophan was determined by means of HPLC and quantified by using an external tryptophan calibration range.

Protein content of each sample was calculated as "the sum of individual amino acid residues (the molecular weight of each amino acid less the molecular weight of water)" (2).

Results and Discussion

Protein and amino acid data were extracted from dotoral theses, journal articles and unpublished reports for raw beef (3 primal cuts), raw lamb (3 primal cuts), raw pork (3 primal cuts), raw chicken (2 cuts), full cream milk, full cream milk powder (reconstituted), plain low fat yoghurt, semi-hard cheeses and five high protein supplements using dfiffrent protein sources as main ingredient.

Table 1: Comparison of the protein by (1) using the respective Jones factors applied to the total nitrogen determined and (2) by using the sum of the amino acid residues

Product decription	Mean protein (g/100g) (N x Jones factor)	Mean protein (g/100g) (∑ Amino Acid Residues)	p-value	Calculated NPCF
Meat: Beef (3,4)				
Beef Prime Rib	18,7	18,1	0,06	6,05
Beef Rump	19,2	17,7		5,76
Beef Shoulder	19,4	16,8		5,41
Meat: Lamb (5)				
Lamb Leg	20,0	16,8	0,001	5,25
Lamb Loin	21,0	18,1		5,38
Lamb Shoulder	19,3	15,9		5,12
Meat: Chicken (5)				
Chicken Breast	22,0	17,7	0,06	5,03
Chicken Drumstick	18,8	15,9		5,29
Meat: Pork (5)				
Pork Loin	21,4	17,4	0,001	5,09
Pork Rump	20,3	16,5		5,08
Pork Shoulder	19,1	15,6		5,10
Dairy: Milk and Yoghurt (6)				
Full cream milk	3,24	2,94	0,11	5,79
Full cream milk powder (reconstituted)	3,20	2,88		5,74
Plain, low fat yoghurt	4,33	3,82		5,63
Dairy : Cheeses (6)				
Gouda cheese	23,9	20,9	0,001	5,58
Cheddar cheese	24,7	21,65		5,59
Protein Sport Supplements (7)				
Whey protein A	71,7	61,8	0,003	5,50
Whey protein B	59,5	49,5		5,31
Whey protein C	30,0	24,6		5,23
Soy Protein	56,7	46,9		4,72
Hydrolysed Beef Protein	69,5	52,2		4,72

Jones factors applied: 6,38 (dairy products and dairy based sport supplements)
5,71 (soy protein based supplements)
6,25 (meat and meat based sport supplements)

For meat (lamb and pork), cheeses and the protein supplements the difference was statistically significant ($p \leq 0.05$). The protein content was over reported when using the respective Jones factor. The difference between the two methods of protein calculation means that the "assumed" nitrogen content of protein is not 16%, but in reality can vary depending on the source of protein. As was previously reported (8,9), NPCF calculated from this data set is lower than the Jones Factors.

Factors affecting NPCF factors include:

- The type of protein;
- Processing methods, such as cooking, can cause protein denaturation;
- Food additives, such as preservatives and flavor enhancers;
- Presence of non-protein nitrogen compounds eg. urea, nucleic acids;
- Nitrogen in collagen, elastin, myoglobin, haemoglobin, high concentration of amino acids with amine side chain;
- Genetic variability and seasonality in foodstuffs.

Conclusion

It is considered that "protein content" is equal to be "amino acid content". However, the indirect analysis using the total nitrogen content of the food multiplied by a conversion factor, will still be used as it is more cost effective and requires less sophisticated analytical instrumentation and skill. Therefore, in the absence and availability of more accurate methods in resource constraint countries to hydrolyse protein to their component amino acid content in order to quantify protein, more discussion is recommended on the updating and application of NPCF to replace Jones factors.

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