ELSEVIER

Food Chemistry 78 (2002) 105–109

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Seed protein contents and nitrogen-to-protein conversion factors for some uncultivated tropical plant seeds

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Received 14 September 2001; received in revised form 2 January 2002; accepted 2 January 2002

Abstract

Seed protein contents and nitrogen-to-protein conversion factors were calculated for 13 lesser-known tropical plant seeds based on total nitrogen and the summation of total amino acids. The total protein content, calculated from total amino acid residue, ranged from 4.90 in *Diospyros mespiliformis* to 32.47 g/100 g fresh weight in *Gliricidia sepium*. Resulting data gave a mean true conversion factor of 4.97 ± 1.07 for the leguminoseae. In some seeds, comparisons indicated significant differences between the protein content based on the traditional factor 6.25 and that from amino acid analysis. An average of 22% of the total seed nitrogen appeared to be non-proteinogenic. In general, a conversion factor of 5.5 appears to provide a much more accurate estimate of the seed proteins. It is concluded that the conversion factor based on the total amino acid, rather than the traditional factor 6.25, is more valid for estimating protein contents in terms of nutritional benefits.

Keywords: Tropical; Uncultivated; Plant seeds; Nitrogen-to-protein; Factors

1. Introduction

Quite a number of lesser-known and wild gathered tropical food crops are currently being subjected to chemical analysis. It is widely believed that there are possibly thousands of lesser-known tropical food plants, which could possibly be developed into new crops and as well be utilized as food or feed. This is especially more important in the sub-Saharan Africa where serious food shortages due to population growth have been widely reported. (Becker 1986, NAS, 1975; Vietemeyer & Janick, 1996). Africa is richly endowed with plant genetic resources, housing an amazing wide genetic diversity. These plant resources, including especially the forests, provide a home for wild relatives of crops, some with useful characters (Franke, 1985; Grivetti, Frentzel, Glinsberg, Howell, & Ogle, 1987; Hutchinson & Dalziel, 1973; Okigbo, 1994). The addition of new sources to the world food supply could measurably reduce malnutrition. Nutritional biochemists are, as a result, continually investigating the possibilities of introducing more food to man and farm animals.

In this context protein sources occupies a special place. The production of protein-rich foods (leguminous seeds and particularly animal products) has been much less efficient in Nigeria and the sub-region. As a result the protein in the diets of the population, derived manly from plant origin, is usually very low in concentration and biological value, even though calorie requirements may be satisfied. In view of the more urgent need of protein sources to combat malnutrition in the tropical countries (FAO, 1964; Friedman, 1996), screening efforts for new crops have focused more on potential sources of concentrated proteins (Evans & Bandemer, 1967; VanEtten, Kwolek, Peters, & Barclay, 1967; Wolf, Hrivnak, Maresova, & Svabova, 1975). For food or feed formulation purposes it is always necessary to determine accurately the nutrient compositions of the various components, particularly unconventional sources in order to achieve optimal utilization of such ingredients in diet. Moreover, accurate estimation of the

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0308-8146/02/$ - see front matter
PII: S0308-8146(02)00105-X

protein contents is necessary so as to narrow down the search to species with the highest potential as protein sources for further research and development.

Crude protein analysis conventionally based on multiplying the total nitrogen (N) (Kjeldalh N) by a factor of 6.25 has been suspect. This factor leads to overestimation due to the fact that a good percentage of the N content is of non-protein origin (Tkachuk, 1969). Some secondary compounds, such as alkaloids, chlorophyll, and certain glycosides contain nitrogen. Various other non-protein N compounds, including amides, free amino acids, non-protein amino acids, nitrogenous fats and ammonium salts, can also occur in plant tissues (McKey, 1974; Rhoades & Cates, 1976; Smith, 1987). Moreover, the value of 6.25 is based on the assumption that protein contains 16% N which, though valid for animal protein, may not be true of plant proteins. Several reports have indeed demonstrated that, for plant tissues, the protein conversion factor is less than 6.25 (Milton & Dintzis, 1981; Mossé, 1990; Yeoh & Wee, 1994). For example, when 6.5 is used for wheat bran or soybean it may overestimate the protein content by 9.7 and 18.8%, respectively (Tkachuk, 1969). For this reason, different conversion factors have been derived and assigned to various food groups and usually listed in food composition tables. Conversion factors recommended for cereals and grain products range between 5.70 and 5.83; for dry grain legumes 5.46–5.71 is recommended; while 5.18–5.46 is for nuts and seeds (FAO, 1982). On the average between species, conversion factors are often close to 5.4 or 5.5 rather than 5.7 or 6.25 as generally accepted (Mossé, 1990). However, the conventional factor 6.25 is still recommended for use in nutritional studies in which diets contain more than one source of nitrogen (Pellett & Young, 1980). This study therefore, sets out to determine the true seed protein contents and to establish the nitrogen-to-protein conversion factors for 13 selected lesser-known non-conventional tropical plant seeds from Nigeria.

2. Materials and methods

Mature seed samples were harvested in Nigeria and treated as previously described (Ezeagu & Ologhobo, 1995). Moisture content was determined by drying at 110 °C using an oven (T 6030, Heraeus Instruments, Hanau, Germany) until reaching weight constancy (at least for 24 h). Total nitrogen was determined by the standard micro-Kjeldahl method (AOAC, 1984) using a digestion apparatus (Kjeldatherm System KT 40, Gerhardt Laboratory Instruments, Bonn, Germany) and a titration system (T110-TR160-TA-TM120, Schott-Geräte GmbH, Hofheim, Germany). Amino acid analyses were carried out according to the recommendations in the report of the joint FAO/WHO expert consultation (FAO/WHO, 1991), hydrolyzed with 6 mol/l hcl (2.5 mg of N/150 ml of HCl, 24 h under reflux by a continuos flow of nitrogen, and after drying (40 °C), washed twice with distilled water to remove residual hydrochloric acid and dried again. Norleucine served as an internal standard. Cysteine and methionine, which are destroyed during the acid hydrolysis, were converted to acid stable derivatives (cysteinic acid and methioninesulfone, respectively) by performic oxidation (Weidner & Eggum, 1966). The oxidized samples were then hydrolyzed with 6N HCL as described above. For tryptophan determination, alkaline hydrolysis was performed according to Rowan, Moughan, and Wilson, (1989) using 4.3 N NaOH in Teflon containers which were flushed with nitrogen and placed in an oven (T 6030, Heraeus Instruments, Hanau, Germany) maintained at 110 °C for 24 h. 5-Methyltryptophan was used as an internal standard. The hydrolyzed samples were stored at 18 °C in citrate buffer at ph 2.2 prior to analysis. Amino acids were analyzed by ion-exchange chromatography with post-column ninhydrin detection using a high performance liquid chromatographic system (System Gold, Beckman Instruments Inc., Fullerton, CA). Released ammonia and 18 common amino acids were measured. Seed protein contents were calculated from the amino acid analysis and expressed as g/100 g fresh wt. samples.

3. Results and discussion

Table 1 shows the moisture, seed protein and N contents, and nitrogen-to-protein conversion factors for the plant seeds. The total protein content calculated from summed individual amino acids ranged from 4.90 in *Diospyros mespiliformis* to 32.47 g/100 g in *Gliricidia sepium*. In using the total amino acid data for this purpose, some contribution by the free protein amino acid as well as the losses of some amino acid residues, such as tryptophan, during acid hydrolysis must be acknowledged. The extent to which free amino acids will influence the overall estimation of seed protein content is likely to vary from plant to plant. It has been shown for 36 grass species that the free amino acids constituted 0.9–12% of the total leaf protein amino acids (Yeoh & Chew, 1976). However, the quantity of free protein amino acids is generally less than 5% of total amino acids (Yeoh & Watson, 1982). In addition, factors such as seed maturity stage and environmental conditions may contribute to changes in protein contents.

Three classes of recovered N contents, relevant to the calculation of nitrogen-to-protein conversion factors, were considered. The first type was the proteinogenic N derived from individual amino acids and it excluded the amide-N of glutamine and asparagine. Proteinogenic N varied widely in the seeds ranging from 1.71 in *Afzelia*

Table 1
Protein content, nitrogen analysis and conversion factors

Species	Moisture (g/100 g)	Protein (g/100 g)	Nitrogen recovery (g/100 g)			Nitrogen-to-protein conversion factors[a]		
			Amino acids	Amino acids $+NH_3$	Kjeldahl nitrogen	k_A	k'_a	k_p
Leguminosaea								
Albizia zygia	7.80	16.26	2.20	3.05	5.26	7.39	5.33	3.09
Afzelia bella	6.81	12.45	1.71	1.99	2.09	7.28	6.26	5.96
Daniellia ogea	9.86	10.68	1.55	1.88	2.00	6.89	5.68	5.34
Enterolobium cyclocarpium	7.71	19.13	2.63	3.33	3.53	7.27	5.74	5.42
Gliricidia sepium	6.77	32.47	4.65	5.60	6.96	6.98	5.80	4.67
Lonchocarpus sericeus	5.49	21.71	2.98	3.44	4.48	7.29	6.31	4.85
Millettia thonningii	4.71	19.66	2.49	3.44	3.60	6.69	5.72	5.46
Prosopis africana	8.67	24.30	3.39	3.87	3.60	7.17	6.28	6.75
Sesbania pachycarpa	6.81	25.00	3.58	4.18	5.27	7.00	5.98	4.49
Pterocarpus osun	5.77	16.65	2.26	2.65	4.56	7.37	6.28	3.65
Mean±SD	7.04±1.54	19.83±6.43	2.74±0.94	3.34±1.09	4.14±1.51	7.13±0.23	5.94±0.34	4.97±1.07
Non-leguminoseae[b]								
Adansonia digitata (Bombaceae)[2]	6.11	16.43	2.30	2.83	2.80	7.14	5.76	5.87
Diospyros mespiliformis(Ebenaceae)	8.99	4.90	0.69	0.84	0.87	7.10	5.83	5.63
Etandraphragma angolense(Meliaceae)	2.63	12.90	1.74	2.09	2.05	7.41	6.17	6.29
Mean±SD	5.91±3.18	11.41±5.91	1.58±0.82	1.91±0.89	7.23±0.17	7.23±0.97	5.92±0.22	5.93±0.33

[a] kA, ratio of protein to amino acid N; k'_A, ratio of protein to nitrogen from amino acids and NH_3; k_P, ratio of protein to Kjeldahl N.
[b] Family name in parenthesis.

bella to 4.65 g/100 g in *G. sepium*. VanEtten et al., (1967) observed similar wide variations among species of leguminosae seeds. The second type was the combination of N derived from individual amino acids and ammonia recovered from acid hydrolysis. Nitrogen determined as ammonia varied between 0.15 in *Diospyros mespiliformis* and 0.95 g/100 g in *G. sepium*, which may be in part due to amides of glutamic and aspartic acids and unstable non-proteinogenic amino acids (Mossé, 1990). Such amides and non-proteinogenic amino acids are known to be present in seeds of several species (VanEtten et al., 1967). The third type of N content was the total N (determined by the micro-Kjeldahl method), which reflects the contribution from both proteins and non-protein sources. Total N varied between 0.8 in *Diospyros mespiliformis* and 6.69 g/100 g in *G. Sepium* and were in each case higher than recovered amino acid and NH_3 N. The percentage of total N recovered as amino acid and NH_3 is usually significantly less than the total Kjeldahl N (Yeoh & Truong, 1996). Possible destruction of amino acids during hydrolysis, possible side reactions during hydrolysis that chelate amino acid N, and presence of nitrogenous compounds that yield neither ammonia nor measured amino acids may be responsible. Also N compounds may be tightly bound within plant cell-wall matrix and are not released during HCl hydrolysis conditions used in the amino acid assay, but concentrated boiling sulphuric acid used in the Kjeldahl method would release such. However, it can be noted that total N seems to be lower for *Prosopis africana* apparently due to some experimental error. Nitrogen calculated from individual amino acids and ammonia might account better for the N recovered from proteins.

A high percentage of N was unidentified [Total N−(Amino acid N + NH_3−N)] in seeds of *Albizia zygia* (42.3%), *Lonchocarpus sericeus* (23.4%), *Prosopsis africana* (32.8%), *Sesbania pachycarpa* (21.06%), *G. sepium* (19.6%) and *Daniellia ogea* (13.8%). Milton and Dinitzis (1981) reported a mean of 20% of total N in a group of tropical plant parts to be non-proteinaceous. As many as 300 non-proteinogenic amino acids are derived from plants and some exhibit toxic properties (Hegarty, 1978; Unterhalt, 1980). Amino acids of non-protein origin such as α, ß-diaminopropionic acid or canavanine 3,4-dihydroxyphenylalanine (L-DOPA) were found in significant amounts in several seeds of leguminous family (Bell, 1963; Mohan & Janardhanan, 1994; Vijayakumaria, Siddhuraju, & Janardhanan, 1993). Other nitrogenous compounds such as nitrates, purines, pyrimidines, alkaloids, B-vitamins and nucleic acids, nitrogenous lipids and glycosides could also contribute to the level of non-proteinogenic nitrogen (McDonald, Edwards, & Greenhalg, 1981). Consequently, as Fig. 1 indicates, some significant differences exists between protein levels as calculated from conversion factor k_p

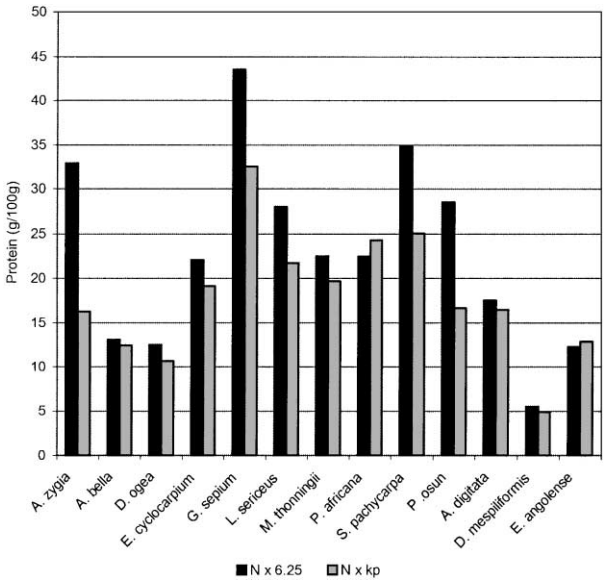

Fig. 1. Comparison of protein values based on factors 6.25 and k_p.

and the traditional factor 6.25. In *Albizia zygia, Pterocapus osun* and *S. pachycarpa* seeds "the true protein" levels were 58.3, 50.4 and 48.5% lower than the "Kjeldahl protein" levels, respectively. Several reports suggest that tropical plant parts generally contain higher proportions of non-proteinogenic N than domesticated plant seed crops (Milton & Dintzis, 1981; Mossé, Huet, & Baudet, 1988; Yeoh & Truong, 1996). Where non-protein nitrogenous compounds occur in appreciable amounts, the traditional conversion factor of 6.25 might well distort protein estimates in terms of nutritional benefits.

Three types of conversion factors were therefore considered. These are (1) k_A, based on the ratio of protein to total N recovered from amino acid analysis, (2) k_A', the ratio of protein to total N recovered from amino acids and ammonia, and (3) k_P, the ratio of protein to Kjeldahl N. As shown in Table 1, k_A values ranged from 6.69 to 7.41, with a mean of 7.13 ± 0.23 for the legumes while k_A' ranged from 5.33 to 6.28, with a mean value of 5.94 ± 0.34 for the legumes. The conversion factor k_P varied between 3.09 in *Albizia zygia* and 6.75 in *Prosopis africana* with a mean of 4.97 ± 1.07 for the legume seeds and 5.93 ± 0.33 for non-legumes. Only factor k_P has a practical value in this study and could be used to estimate the protein content from Kjeldahl N analysis. The k_P values compares favourably with the range of 3.28–6.31 reported for a group of plant species (Milton & Dintzis, 1981; Mossé, 1990; Yeoh & Wee, 1994). The mean value of 4.97 ± 1.07 recorded for the legumes is also close to the range of 5.18–5.71 recommended for grain legumes, nuts and seeds (FAO, 1982).

This study has shown again that the traditional practice of using 5.7 or 6.25 as factors in calculating protein content was based on an incorrect assumption and erroneous conclusions, because not all the N as determined by the Kjeldahl method are of protein origin. A correction factor is therefore needed to account only for the nitrogen found as protein amino acid, which represents the "true protein" contents (Pellet & Young, 1980; Tkachuk, 1969). The factor k_P has been named the corrected conversion factor and has indeed the advantage of taking into account non-protein nitrogen (Sosulski & Holt, 1980). Corrected crude protein values based on the ratio of amino acid N recovery data to total N (Petzke, Ezeagu, Proll, Akinsoyinu, & Metges, 1997) are closely similar to that from the summation of individual amino acid residues in this report. Therefore, to obtain a better estimate of the protein contents of these plant seeds, the result suggests the use of the derived mean k_P values of 5.0 and 5.9 for the legumes and non-legumes, respectively. However, in general, a k_P of 5.5 should provide a good estimate of the seed protein contents for the seed samples.

Acknowledgements

I.E.Ezeagu was a DAAD (Deutscher Akademischer Austauschdienst, Bonn, Germany) scholar at the German Institute of Human Nutrition, Arthur-Scheunert-Allee, 114–116, D14558 Bergholz-Rehbrücke, Germany. The financial support is gratefully acknowledged. Above plant seeds can be available from the author.

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