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Leaf protein contents and nitrogen-to-protein conversion factors for 90 plant species

Hock-Hin Yeoh & Yeow-Chin Wee

Department of Botany, National University of Singapore, Kent Ridge, Singapore 0511

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The leaf protein contents of 90 plants covering taxonomically diverse groups ranged from 0·2 to 7·5 g% fr. wt. The nitrogen-to-protein conversion factors (k_A') based on nitrogen recovered from amino acid analyses ranged from 5·15 to 5·93, reaffirming that the traditional factor of 6·25 is not valid for plant proteins. A more practical conversion factor (k_P) based on the ratio of protein from amino acid data to Kjeldahl nitrogen varied from 3·28 to 5·16. Variations in both leaf protein contents and the conversion factors to some extent correlated with the taxonomic groupings of the plants. For a good estimate of the leaf protein content from Kjeldahl nitrogen, factor k_P, established using plants sampled from the same taxonomic group, should be used. However, for plants in general, a k_P of 4·43 should provide a reasonably good estimate of the protein content.

INTRODUCTION

Leaves are a potential source of proteins. They have both actual and potential values as animal feed and in the production of unconventional protein food (Pirie, 1986). Many tropical plant species are potentially useful in this respect and may not have been analysed for their protein content. It is therefore useful to have a quick and reasonably accurate method of estimating the protein content. The total nitrogen analysis using the Kjeldahl method is still widely favoured for crude protein estimation but its accuracy is dependent on the nitrogen-to-protein conversion factors used. There have been a few reports showing that the traditional protein conversion factor of 6·25 is not valid for plant materials (Milton & Dintzis, 1981; Handley *et al.*, 1989; Mosse, 1990). So far, the conversion factor reported for only a small number of plant species ranged from 3·7 to 6·0 (Milton & Dintzis, 1981; Handley *et al.*, 1989).

Thus we have set out to determine the leaf protein content and establish the nitrogen-to-protein conversion factors for a wide ranging and taxonomically diverse group of plants. Bearing in mind that taxonomic schemes have predictive capabilities in various contexts and in our previous studies on plant proteins (Yeoh & Watson, 1981, 1982; Yeoh *et al.*, 1986, 1992), the plants used in this study have been selected to represent the major taxonomic groups of the plant kingdom. Moreover, results from such a study can help us assess how far a knowledge of taxonomy is helpful in identifying variations in leaf protein contents and the nitrogen-to-protein conversion factors.

MATERIALS AND METHODS

Freshly harvested mature leaves were used for all analyses. Leaf samples were collected from plants grown in the Botany Department garden, the Singapore Botanic Gardens and elsewhere in Singapore. Total nitrogen was determined using the micro-Kjeldahl technique (Bailey, 1967) with 1 g fr. wt leaf. For amino acid analysis, finely cut leaf blades (100–200 mg) were hydrolysed in 0·5 ml 3N mercaptoethane sulphonic acid in a sealed tube at 110°C for 22 h according to Yeoh *et al.* (1986), then analysed using the Beckman amino acid analyser 119CL. For total nitrogen determination, four samples were analysed whereas for amino acid determination duplicate analyses were carried out. Leaf protein contents were calculated from the amino acid analyses and expressed as g% fr. wt. Moisture was determined as described in Bradbury and Holloway (1988). Significant differences reported are at the 5% probability level.

RESULTS AND DISCUSSION

The plants used in this study covered the three major plant phyla, namely the Pteridophyta, Gymnospermae and Angiospermae. We have also analysed the angiosperm data against the super-orders of Dahlgren's

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Table 1. Moisture, protein and nitrogen analyses of leaves of Pteridophyta and Gymnospermae

Species	Moisture (g% fr. wt)	Protein (g% fr. wt)	Nitrogen recovery			Nitrogen-to-protein conversion factors		
			Amino acids	Amino acids and NH_3 (g% fr. wt)	Kjeldahl nitrogen	k_A	k'_A	k_p
Pteridophyta								
Angiopteris evecta	84·7	0·94	0·15	0·16	0·19	6·15	5·71	4·91
Asplenium nidus	80·5	0·99	0·16	0·17	0·20	6·19	5·82	4·95
Bolbitis heteroclita	73·9	2·83	0·46	0·52	0·55	6·11	5·69	5·14
Cibotium barometz	64·8	3·61	0·59	0·62	0·80	6·11	5·81	4·52
Cyathea latebrosa	71·1	2·21	0·36	0·38	0·50	6·15	5·82	4·42
Davallia denticulata	74·2	2·23	0·36	0·38	0·47	6·25	5·83	4·72
Dicranopteris linearis	55·9	2·44	0·39	0·42	0·59	6·19	5·85	4·16
Lygodium microphyllum	70·8	1·83	0·30	0·32	0·43	6·11	5·78	4·24
Nephrolepis biserrata	85·6	1·15	0·19	0·20	0·26	6·14	5·77	4·47
Trichomanes javanicum	77·6	2·02	0·33	0·36	0·40	6·14	5·67	5·04
Taxonomic mean	73·9	2·03	0·33	0·35	0·44	6·15	5·78	4·66
Gymnospermae								
Araucaria columnaris	52·5	2·65	0·43	0·47	0·81	6·16	5·62	3·28
Cycas rumphii	67·0	3·88	0·63	0·69	0·90	6·17	5·62	4·33
Gingko biloba	78·7	3·10	0·50	0·54	0·64	6·18	5·77	4·81
Gnetum gnemon	65·1	4·65	0·74	0·81	1·10	6·25	5·76	4·24
Pinus merkusii	61·1	1·91	0·31	0·34	0·42	6·12	5·64	4·50
Podocarpus polystachyus	47·4	3·44	0·56	0·60	0·84	6·19	5·75	4·11
Taxonomic mean	62·0	3·27	0·53	0·58	0·79	6·18	5·69	4·21

k_A, ratio of protein to amino acid nitrogen; k'_A, ratio of protein to nitrogen from amino acids and ammonia; k_p, ratio of protein to Kjeldahl nitrogen.

scheme (Dahlgren, 1980) and have superimposed on Dahlgren's dicotyledon super-orders the main division into Crassinucelli and Tenuinucelli advocated by Young and Watson (1970).

Tables 1–3 give the moisture, leaf protein and nitrogen contents, and nitrogen-to-protein conversion factors for the 90 plants covering the pteridophytes, gymnosperms and angiosperms. The total leaf protein amino acid content of these plants represent not only amino acids derived from proteins but also those in the free form. Thus in the calculation of protein content from total leaf amino acid data, contribution by the free protein amino acids must be acknowledged. The extent to which the free amino acids will influence the overall estimation of leaf protein content is likely to vary from plant to plant. It was shown for 36 grass species that the free amino acids constituted 0·9–12 % of the total leaf protein amino acids (Yeoh & Watson, 1982). However, the quantity of free protein amino acids is generally less than 5% of total leaf amino acids (Yeoh & Chew 1976; Yeoh & Watson, 1982). In interpreting the results one must be cautioned that many factors, such as plant-leaf age, stage of growth and environmental conditions could contribute to changes in the quantity and quality of leaf proteins, amino acid compositions and other nitrogenous compounds (Smith, 1976; Yeoh & Watson, 1982; Yeoh & Paul, 1989).

A large variaticn in protein content was observed, ranging from 0·2 g% fr. wt in *Dischidia nummularia* to 7·45 g% fr.wt in *Adenanthera pavonia*. Group by group comparisons showed that the Gymnospermae and Angiospermae had protein contents (3·3±1·0 g% fr. wt and 3·3±1·7 g% fr. wt, respectively) significantly higher than that of the Pteridophyta (2·0±0·8 g% fr. wt). Within the Angiospermae, the dicotyledonous plants exhibited significantly higher protein content (3·8±1·7 g% fr. wt) than the monocotyledonous plants (1·7±0·8 g% fr.wt)(Tables 2 and 3). Leaves of grasses were reported to be poor in protein, averaging 2·2±1·0 g% fr. wt (Yeoh & Watson, 1982), similar in range to those reported here for the monocotyledonous plants.

Within the Dicotyledonae, differences in leaf protein contents were observed for some of Dahlgren's super-orders; the Malviflorae, Violiflorae and Fabiflorae exhibited higher leaf protein contents (4·4 g% fr. wt) than those of Magnoliflorae, Theiflorae and Gentianiflorae (3·5 g% fr. wt; Table 2). With respect to Young and Watson's scheme (Young & Watson, 1970), the crassinucellate group exhibited a protein content (4·0±1·7 g% fr. wt) significantly higher than that of the tenuinucellate group (2·9±1·3 g% fr. wt). Even among the closely related crassinucellate members, the legumes yielded protein content (5·2±1·6 g% fr.wt) significantly higher than those of the caryophylloids (4·0±1·7 g% fr. wt) and magnolioids (3·5±1·1 g% fr. wt). Although many factors such as the physiology of the plants and environmental conditions, could affect the levels of proteins in the leaves, it is noteworthy that differences are detected at different levels of taxonomic hierarchy.

Three types of nitrogen contents relevant to the calculation of nitrogen-to-protein conversion factors were considered. The first type was nitrogen derived

Table 2. Moisture, protein and nitrogen analyses of the leaves of Angiospermae—Subclass Dicotyledonae

Species	Moisture (g% fr. wt)	Protein (g% fr. wt)	Nitrogen recovery			Nitrogen-to-protein conversion factors		
			Amino acids	Amino acids and NH_3 (g% fr. wt)	Kjeldahl nitrogen	k_A	k'_A	k_p
Magnoliflorae[1]								
Annona squamosa[b]	51·3	3·53	0·57	0·62	0·93	6·16	5·65	3·80
Michelia champaca[b]	54·7	3·66	0·59	0·63	0·90	6·19	5·78	4·06
Myristica fragrans[b]	62·6	2·50	0·41	0·44	0·55	6·13	5·73	4·53
Taxonomic mean	56·2	3·23	0·52	0·56	0·79	6·16	5·72	4·13
Nymphaeiflorae[1]								
Piper nigrum	65·9	1·74	0·28	0·30	0·44	6·19	5·73	3·99
Caryophyliflorae[1]								
Amaranthus blitum[a]	88·9	2·63	0·42	0·46	0·55	6·26	5·76	4·74
Mirabilis jalapa[a]	89·3	1·58	0·25	0·27	0·35	6·25	5·80	4·55
Polygoniflorae[1]								
Antigonon leptopus[a]	74·2	3·58	0·58	0·63	0·92	6·13	5·72	3·89
Malviflorae[1]								
Artocarpus heterophyllus[a]	68·8	3·63	0·59	0·63	0·85	6·20	5·80	4·28
Bixa orellana[a]	58·3	4·47	0·72	0·77	1·02	6·24	5·84	4·38
Ficus religiosa[a]	68·9	4·04	0·65	0·70	0·90	6·22	5·80	4·49
Hevea brasiliensis[c]	56·1	5·81	0·94	1·03	1·26	6·16	5·65	4·61
Muntingia calabura[c]	59·8	5·93	0·96	1·02	1·31	6·20	5·84	4·53
Sida rhombifolia[c]	56·4	3·54	0·57	0·61	0·80	6·21	5·77	4·41
Taxonomic mean	61·4	4·57	0·74	0·79	1·02	6·20	5·78	4·45
Violiflorae[1]								
Carica papaya[b]	79·2	3·12	0·51	0·54	0·70	6·17	5·77	4·45
Passiflora foetida[b]	70·8	5·32	0·85	0·93	1·11	6·25	5·73	4·79
Salix babylonica[b]	69·3	4·95	0·79	0·85	1·08	6·23	5·86	4·57
Taxonomic mean	73·1	4·46	0·71	0·77	0·96	6·22	5·79	4·60
Theiflorae[1]								
Cratoxylon formosum[b]	58·1	1·93	0·31	0·34	0·41	6·15	5·67	4·68
Mesua ferrea[b]	47·8	2·52	0·41	0·44	0·55	6·18	5·74	4·55
Nepenthes raffiesiana[a]	78·6	1·55	0·25	0·27	0·33	6·20	5·73	4·63
Taxonomic mean	61·5	2·00	0·32	0·35	0·43	6·17	5·71	4·62
Primuliflorae[1]								
Manikara zapota[e]	47·4	3·13	0·51	0·55	0·67	6·09	5·67	4·49
Rosiflorae[1]								
Casuarina equisetifolia[b]	61·0	2·95	0·48	0·51	0·65	6·15	5·77	4·52
Kalanchoe pinnata	88·4	0·72	0·12	0·13	0·17	6·17	5·46	4·34
Fabiflorae[1,d]								
Acacia auriculiformis	61·6	3·59	0·58	0·63	0·87	6·18	5·74	4·10
Adenanthera pavonina	57·1	7·45	1·21	1·32	1·44	6·16	5·64	5·12
Albizia falcataria	56·3	4·46	0·72	0·77	1·23	6·18	5·80	3·62
Baphia nitida	61·5	6·31	1·01	1·08	1·49	6·24	5·83	4·24
Cassia biflora	59·5	5·42	0·88	0·94	1·16	6·15	5·78	4·68
Crotalaria retusa	74·2	3·09	0·50	0·53	0·74	6·19	5·79	4·20
Delonix regia	52·4	5·65	0·91	0·97	1·51	6·24	5·84	3·75
Lucaena leucocephala	62·8	7·01	1·12	1·30	1·89	6·24	5·39	3·72
Millettia atropurpurea	52·6	4·24	0·68	0·73	0·87	6·23	5·80	4·88
Mimosa pudica	72·8	7·02	1·13	1·22	1·40	6·21	5·78	5·03
Parkia javanica	65·8	3·52	0·57	0·61	0·83	6·20	5·75	4·23
Psophocarpus tetragonolobus	70·2	6·75	1·09	1·16	1·57	6·21	5·82	4·29
Pterocarpus indicus	60·0	6·47	1·02	1·09	1·55	6·32	5·93	4·17
Saraca thaipingensis	59·7	3·32	0·54	0·58	0·80	6·21	5·76	4·13
Sesbania grandiflora	70·5	6·42	1·03	1·11	1·55	6·26	5·77	4·15
Tamarindus indica	69·1	2·75	0·45	0·48	0·61	6·16	5·70	4·49
Taxonomic mean	62·9	5·22	0·84	0·91	1·22	6·21	5·76	4·30

contd.

Table 2—*contd.*

Species	Moisture (g% fr. wt)	Protein (g% fr. wt)	Nitrogen recovery			Nitrogen-to-protein conversion factors		
			Amino acids	Amino acids and NH_3 (g% fr. wt)	Kjeldahl nitrogen	k_A	k'_A	k_p
Myrtiflorae[1]								
Lagerstroemia speciosa	74·9	2·28	0·37	0·40	0·47	6·17	5·75	4·88
Melastoma malabathricum	65·6	4·24	0·69	0·73	0·92	6·17	5·82	4·61
Rutiflorae[1]								
Averrhoa carambola[a]	67·0	4·72	0·76	0·81	0·99	6·23	5·85	4·75
Mangifera indica[c]	56·3	3·17	0·51	0·59	0·72	6·18	5·76	4·39
Murraya koenigii[c]	71·1	4·06	0·65	0·70	1·04	6·25	5·76	3·92
Nephelium lappaceum[c]	57·0	2·79	0·45	0·49	0·63	6·19	5·74	4·46
Malpighia coccigera	56·5	2·77	0·46	0·51	0·76	5·99	5·43	3·65
Taxonomic mean	61·6	3·50	0·57	0·62	0·83	6·19	5·71	4·23
Asteriflorae[2]								
Mikania cordata[g]	83·5	1·78	0·29	0·31	0·38	6·20	5·77	4·69
Solaniflorae[2]								
Ipomoea aquatica[e]	89·5	3·10	0·50	0·54	0·62	6·19	5·74	4·97
Merremia tridentata[e]	80·0	2·18	0·35	0·38	0·45	6·24	5·76	4·85
Gentianiflorae[2]								
Adina rubescens[e]	57·5	3·53	0·57	0·61	0·78	6·17	5·80	4·54
Cebera odollum[e]	77·9	3·18	0·51	0·55	0·69	6·19	5·77	4·58
Dischida nummularia[e]	91·8	0·20	0·03	0·04	0·05	6·18	5·15	4·00
Morinda citrifolia[e]	75·9	3·45	0·56	0·60	0·70	6·14	5·74	4·90
Taxonomic mean	75·8	2·59	0·42	0·45	0·56	6·17	5·62	4·51
Lamiiflorae[2]								
Lantana aculeata[f]	62·6	5·47	0·87	0·93	1·09	6·28	5·86	5·02
Mentha arvensis[f]	84·0	1·73	0·28	0·30	0·35	6·20	5·75	5·00
Spathodea campanulata[f]	70·7	3·98	0·64	0·68	0·82	6·20	5·82	4·87
Taxonomic mean	72·4	3·73	0·60	0·64	0·75	6·23	5·81	4·96
Corniflorae[2]								
Cantella asiatica[h]	83·5	2·51	0·40	0·43	0·68	6·23	5·86	3·68
Taxonomic mean for subclass	67·3	3·78	0·61	0·66	0·86	6·19	5·74	4·43

Scheme after Dahlgren (1980). 1 and 2 denote Young and Watson's crassinucelli and tenuinucelli, superscript letters follow Young and Watson's classification as caryophylloids (*a*), magnolioids (*b*), celestroids (*c*), Leguminosae (*d*), asclepioids (*e*), acanthoids (*f*), Compositae (*g*) and Umbelliferae (*h*), respectively (Young & Watson, 1970). k_A, ratio of protein to amino acid nitrogen; k'_A, ratio of protein to nitrogen from amino acids and ammonia; k_p, ratio of protein to Kjeldahl nitrogen.

from individual amino acids and it excluded the amide–N of glutamine and asparagine. For lack of specific amide–N determination, the total ammonia from acid hydrolysis could be used to approximate the amide–N of glutamine and asparagine (Mosse, 1990). However, it must be cautioned that the unbound forms are always present in the leaves. Nonetheless, this second type nitrogen calculated from individual amino acids and ammonia recovered from acid hydrolysis might account better for the nitrogen recovered from proteins. For the 90 plant species investigated, the nitrogen recovered from amino acids alone averaged 93% of the nitrogen from amino acids and ammonia, suggesting that the amide–N contributed not more than 7% of the total protein nitrogen. The third type of nitrogen content (Kjeldahl nitrogen) reflected the contribution from both proteins and non-protein sources. Assuming the recovery of nitrogen from amino acid analysis was close to 100 %, the data then showed that, on the average, 76% of the nitrogen in these plants came from protein sources.

Bearing the above in mind, we considered three types of conversion factors. These are (i) k_A, the ratio of protein to total nitrogen from amino acids (excluding the amide–N), (ii) k_A', the ratio of protein to total nitrogen recovered from amino acids and ammonia, and (iii) k_P, the ratio of protein to Kjeldahl nitrogen. The values for these conversion factors were independent of the nitrogen or protein content of the plant material (Tables 1, 2 and 3).

Table 3. Moisture, protein and nitrogen analyses of leaves of the Angiospermae—Subclass Monocotyledonae

Species	Moisture (g% fr. wt)	Protein (g% fr. wt)	Nitrogen recovery			Nitrogen-to-protein conversion factors		
			Amino acids	Amino acids and NH_3 (g% fr. wt)	Kjeldahl nitrogen	k_A	k'_A	k_p
Alismatiflorae								
Sagittaria sagittifolia	81·2	2·17	0·35	0·39	0·53	6·14	5·52	4·08
Ariflorae								
Alocasia macrorrhiza	81·3	2·95	0·48	0·51	0·59	6·14	5·73	4·97
Diffenbachia reginae	83·8	1·95	0·32	0·34	0·43	6·16	5·75	4·55
Scindapsus aureus	89·0	0·91	0·15	0·17	0·24	6·18	5·46	3·83
Taxonomic mean	84·7	1·94	0·32	0·34	0·42	6·16	5·65	4·45
Lilliflorae								
Aranda 'Christine'	83·0	0·59	0·10	0·11	0·12	6·14	5·57	4·76
Dendrobium crumenatum	91·5	0·52	0·08	0·09	0·13	6·22	5·57	4·02
Eichhornia crassipes	80·8	1·93	0·31	0·34	0·52	6·18	5·75	3·68
Gloriosa superba	82·0	2·79	0·45	0·48	0·58	6·16	5·79	4·77
Haemanthus multiflorus	91·9	1·31	0·21	0·24	0·31	6·16	5·50	4·23
Tacca cristata	81·6	1·92	0·63	0·67	0·91	6·11	5·72	4·23
Taxonomic mean	85·1	1·51	0·30	0·32	0·43	6·16	5·65	4·28
Zingiberiflorae								
Languas galanga	77·0	2·10	0·34	0·34	0·43	6·11	5·71	4·25
Musa paradisiaca	85·5	2·12	0·34	0·37	0·41	6·17	5·72	5·16
Commeliniflorae								
Ananas comosus	85·9	0·34	0·06	0·06	0·08	6·17	5·45	4·38
Cyperus aromaticus	65·3	2·36	0·38	0·41	0·52	6·24	5·79	4·58
Rhoeo spathacea	91·2	0·44	0·07	0·08	0·10	6·13	5·53	4·43
Taxonomic mean	80·8	1·05	0·17	0·18	0·23	6·18	5·59	4·46
Areciflorae								
Archontophoenix alexandriae	69·0	2·32	0·38	0·41	0·54	6·12	5·65	4·33
Areca catechu	76·8	2·46	0·40	0·43	0·63	6·15	5·78	3·91
Pandanus odorus	80·9	1·82	0·29	0·32	0·40	6·24	5·73	4·57
Taxonomic mean	75·6	2·20	0·36	0·39	0·52	6·17	5·72	4·27
Taxonomic mean for subclass	82·1	1·72	0·30	0·32	0·42	6·16	5·65	4·37

Scheme after Dahlgren (1980). k_A, ratio of protein to amino acid nitrogen; k'_A, ratio of protein to nitrogen from amino acids and ammonia; k_p, ratio of protein to Kjeldahl nitrogen.

The conversion factor k_A had values ranging from 5·99 to 6·32, with an average of 6·18±0·05 for the 90 plants. Group by group comparison showed that the three major taxonomic groups gave very similar values for k_A. Nonetheless, the monocotyledonous plants as a group showed a lower conversion value (6·16±0·04) than that for the dicotyledonous plants (6·19±0·05; Tables 2 and 3). Differences in the leaf amino acid compositions of these two major groups could account for the difference in the conversion factor (Yeoh *et al.*, 1986, 1992).

The conversion factor k_A' ranged from 5·15 to 5·93 for the 90 plants (Tables 1, 2 and 3). The average value was 5·72±0·12. Comparison by taxonomic grouping showed closely similar k_A' values for the Gymnospermae and Angiospermae (5·69±0·07 and 5·72±0·13, respectively). k_A' for Pteridophyta, on the other hand, was higher (5·78±0·10). Within the Angiospermae, the k_A' for the Dicotyledonae (5·74±0·13) was higher than that of the Monocotyledonae (5·65±0·13). It is interesting that the values for k_A and k_A' were different from the traditional factor of 6·25. However, this is not surprising as the factor of 6·25 was derived from animal protein composition studies.

The conversion factor k_P has a practical value. It permits rapid estimation of protein content from Kjeldahl nitrogen analysis. From our study of 90 plants, this factor ranged from 3·28 to 5·16, with an average of 4·43±0·40. This value is closely similar to that reported by Milton and Dintzis (1981). Based on a taxonomic mean value, the k_P for Gymnospermae was 4·21±0·52, that for Pteridophyta was 4·66±0·34 and for Angiospermae it was 4·41±0·39 (Tables 1, 2 and 3). Variations were also observed in the k_P values among the major taxonomic groups of the Angiospermae but they were not significantly different (Tables 2 and 3).

From these data it is clear that the factor 6·25 is unsuitable for estimating leaf protein contents of plants from Kjeldahl nitrogen analyses. To obtain a better estimate of protein content, the results suggest the use of k_P values derived from related taxonomic groups. However, a k_P of 4·43 should give a reliable estimate of the leaf protein content for plants in general.

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