Variation in Nitrogen-to-Protein Conversion Factor for Peanut

J. B. Misra[1]

ABSTRACT

For peanut, a factor of 5.46 is used for converting nitrogen concentration into protein concentration because the peanut proteins arachin and conarachin contain 18.31% nitrogen. Using published reports on the amino acid composition of peanut genotypes, the nitrogen-to-protein conversion factors (NPCF) for arachin and conarachin, as well as for whole peanut kernel were calculated. The value of NPCF for arachin varied between 5.271 and 5.563 and that for conarachin from 5.076 to 5.496. The location of crop growth may significantly affect the value of NPCF for whole kernels. For the samples of various genotypes grown at various locations, the values of NPCF varied between 5.3 and 5.7. Thus, the protein content obtained by using the conventional factor of 5.46 would have a precision of ± 0.7% for samples having 3.5% N (approx. 18% protein). As the nitrogen content increases, the precision decreases to reach ± 1.1% for samples having 5.5% N (approx. 30% protein). This implies that the protein contents of peanut calculated on the basis of a fixed conversion factor would be significantly different only when they differ by 1.4 to 2.2% in the calculated values. Therefore, use of a fixed NPCF for comparison of concentration of protein in samples emanating from breeding or agronomic trials may be erroneous, especially when samples differ only marginally in their protein concentrations. Research will be required to determine the relationship between the nitrogen concentration of peanut kernel samples and their protein concentration.

Key Words: *Arachis hypogaea* L., groundnut.

Peanut is unique among oilseeds in that it also can be consumed directly as an item of food. In addition to oil, peanut kernels contain about 18% protein. The peanut cake, which is left behind after expulsion of oil, contains about 30% protein. Conventionally, the protein concentration of foods and feeds is obtained by multiplying the total nitrogen concentration by a nitrogen-to-protein conversion factor (NPCF). This practice originated from early research on proteins of animal origin, which contain approximately 16% nitrogen (100 ÷ 16 = 6.25). This assumption was, however, found incorrect and more accurate NPCF for different plant proteins subsequently were proposed with values varying from 5.18 to 6.25 (Jones, 1931). Later, a N:P Conversion Factor Committee of the Assoc. of Official Chemists (USA) concluded that accurate factors for conversion of nitrogen concentration into protein concentration do not exist (Baker, 1982). However, in the absence of any other practical method, the following factors printed in Method 14.067 in AOAC (1984) are used—5.7 for wheat, 5.18 for almonds, 5.46 for peanut and Brazil nut; 5.30 for coconut and tree nuts, 6.38 for dairy products, and 6.25 for all other plant and animal proteins.

The FAO (1970), however, continues to use a universal factor of 6.25. The value of 5.46 for peanut was derived because arachin, the major seed protein of peanut, contains 18.31% nitrogen (Jones, 1931). As pointed out earlier, NPCF based on nitrogen concentration of major seed proteins is erroneous due to variability of these proteins (e.g., wheat glutelin, barley hordein, oat prolamin, peanut arachin and conarachin, etc.). Tkachuk (1969) suggested that it would be appropriate to express total protein concentration in cereals and oilseeds as the sum of all proteins and small amounts of peptides and amino acids that also are present in the seeds. Accordingly, more precise NPCF conversion factors have been calculated for several commodities (Sosulski and Imafidon, 1990). According to Mossé (1990), NPCF for a given grain or oilseed may not necessarily be an inverse of N percentages of total proteins. Furthermore, the value of NPCF may vary with the concentration of nitrogen in grains. Thus, the true NCPF for rice grains may range from 5.1 (for low N content rice) to 6.0 (for high N content rice), whereas the NCPF for barley, soybean, and sorghum is not affected significantly by the nitrogen contents of the grain (Mossé, 1990). The objective of this study was to determine the variability in NPCF for peanut.

Materials and Methods

Definition of Nitrogen-to-Protein Conversion Factor (NPCF). The true NPCF has been defined by Mossé (1990) as the ratio of actual seed proteins to total N recovered from 20 amino acids. This includes amide-N of glutamine and

[1]Nat. Res. Centre for Groundnut, Ivanagar Rd., Post Box No. 5, Junagadh-362001, Gujarat, India (email: misra@nrcg.guj.nic.in).

asparagine, which is released as ammonia during the acid hydrolysis.

Data Required for Calculation of NPCF for Peanut. For calculation of NPCF, total amino acid analysis should determine the total quantity of each of the 20 amino acids whether present in bound (protein or peptides) or free form (as the free amino acids also contribute to the protein value). However, during digestion in 6 N HCl, which liberates protein-bound amino acids, tryptophan is destroyed. Also, the amide-N from glutamine and asparagine is released as ammonia, converting these amides into glutamic acid and aspartic acid, respectively. Therefore, in most published reports on amino acid analysis of peanut, data on tryptophan are not available and those on ammonia have not been reported. There are, however, a few reports on amino acid analysis of whole seeds (Young, 1979; Hovis *et al.*, 1982) and one on arachin and conarachin (Dawson and McIntosh, 1973) which contain values for ammonia but not for tryptophan.

Accounting for Missing Value of Tryptophan. Tryptophan content of peanut proteins ranges between 1.05 to 1.41 g/16 N (Amaya *et al.*, 1977). Accordingly, for testing the effect of inclusion of values of tryptophan content on the NPCF, sample calculations were made by including tryptophan at the levels of 0, 0.5, 1, and 1.5%. Since the inclusion of any of the stated levels of on tryptophan did not affect the value of NPCF, further calculations used tryptophan value at 1% only.

Calculation of NPCF. The methods outlined by Tkachuk (1969) and suggestions of Mossé (1990) were taken into consideration for calculating the NPCFs as follows:

1. The total weight of hydrolyzed protein was determined by summing the weights of all amino acids while including tryptophan at 1% level. The weight of ammonia was not taken into account while calculating the total weight of amino acids for the reasons explained by Mossé (1990).
2. The total number of moles of water consumed during hydrolysis of protein was taken to be equal to the number of moles of amino acids liberated during the hydrolysis.
3. The weight of nitrogen in protein was calculated by summing the quantities of nitrogen contributed by each amino acid, including that released as ammonia.
4. The weight of copolymerized amino acids (protein) was taken to be equal to total weight of amino acids, minus weight of water consumed during hydrolysis.
5. NPCF was calculated by dividing the weight of copolymerzied amino acids by the weight of nitrogen present in them.

Amino acid data were entered into a Microsoft Excel spreadsheet, which calculated NPCF. A model calculation is shown in Table 1.

In this paper, the data on amino acid analysis of whole peanut protein published by Young (1979) and Hovis *et al.* (1982) and that of arachin and conarachin published by Dawson and McIntosh (1973) have been used for calculating new NCPFs for peanut.

Table 1 A model exercise for calculating NPCF by using the amino acid analysis data published by Hovis *et al.* (1982).

	Amino acid composition	Formula weight of amino acid	Nitrogen	Amino acid	Nitrogen
	A	B	C	D = (A+B)	E = (D x C)
	g/100 g	atomic mass unit	g/mol amino acid	mol/100 g sample	g/100 g sample
ASP	12.43	133.104	14.007	0.093	1.308
THR	2.66	119.120	14.007	0.022	0.313
SER	5.20	105.093	14.007	0.049	0.693
GLU	16.74	147.130	14.007	0.114	1.594
PRO	6.07	115.132	14.007	0.053	0.738
GLY	6.32	75.067	14.007	0.084	1.179
ALA	4.12	89.094	14.007	0.046	0.648
CYS	1.37	121.154	14.007	0.011	0.158
VAL	4.18	117.147	14.007	0.036	0.500
MET	1.43	149.207	14.007	0.010	0.134
ILE	3.86	131.174	14.007	0.029	0.412
LEU	6.92	131.174	14.007	0.053	0.739
TYR	4.04	181.191	14.007	0.022	0.312
PHE	5.44	165.191	14.007	0.033	0.461
HIS	3.19	155.156	42.021	0.021	0.864
LYS	3.47	146.189	28.014	0.024	0.665
ARG	11.98	174.202	56.028	0.069	3.853
TRY	1.00	204.228	28.014	0.005	0.137
NH_4	0.59	18.038	14.007	0.033	0.458
Total	100.42[a]			0.774	15.167

Calculation of NPCF:

Wt of water molecules added during hydrolysis (total of D x 18) = 13.933.

Wt of copolymerized amino acids or protein (total of A - 13.9334) = 86.487.

Wt of nitrogen in protein (copolymerized amino acids) = 15.167.

NPCF (wt of protein ÷ wt of nitrogen) = 5.702.

[a]Does not include weight of ammonia.

Results and Discussion

Effect of Genotypes on NPCF. The factors calculated for six genotypes from the amino acid analysis of Hovis *et al.* (1982) were in the range of 5.289 to 5.702 (maximum for Tennessee and minimum for Florunner) with 5.486 and 3.50% as the values for mean and coefficient of variation, respectively. The values for the remaining four genotypes were 5.656 for cv. Tifspan, 5.616 for cv. Spancross, 5.348 for F 334-A-B-14, and 5.302 for cv. White Maneyma.

The values of NPCF calculated from the amino acid analysis of eight genotypes grown at seven locations (Young, 1979) ranged from 5.321 (cv. GK 19) to 5.661 (Spancross), both of which grown at College Station, TX (Table 2). However, on the basis of values of NPCF averaged over seven locations, the genotypic differences narrowed to 5.468 for TP 1025 to 5.532 for Spancross. The genotypic differences in values calculated using the data of Young (1979) were not significant statistically.

Effect of Location on NPCF. The analysis of variance of NPCF values indicated significant effects of location of crop growth at the 1% level of significance (Table 2). When comparing LSD for 5% level of significance, the highest value of NPCF (5.579) was obtained for peanut grown at Suffolk, VA, which was not different than the value obtained for Perkins, OK, but higher than the values obtained for Gainsville, FL; Headland, AL; Lewiston, NC; College Station, TX; and Tifton, GA. The lowest value (5.448) was obtained for Tifton and, while

Table 2. Effect of genotype and location on NPCF (calculated on the basis of amino acid analysis by Young (1979).

Location	Genotype								Mean
	Comet	Spancross	Spanhoma	Starr	Tifspan	TP 716-2-1	GK 19	TP 1025	
Suffolk, VA	5.582	5.583	5.555	5.591	5.590	5.605	5.582	5.542	5.579
Lewiston, NC	5.457	5.473	5.461	5.460	5.455	5.466	5.456	5.463	5.461
Tifton, GA	5.484	5.440	5.492	5.454	5.363	5.459	5.460	5.435	5.448
Headland, AL	5.464	5.460	5.460	5.434	5.473	5.503	5.482	5.479	5.469
Gainesville, FL	5.556	5.523	5.431	5.467	5.568	5.580	5.489	5.430	5.505
College Station, TX	5.447	5.661	5.569	5.475	5.370	5.407	5.321	5.382	5.454
Perkins, OK	5.541	5.585	5.574	5.513	5.567	5.554	5.557	5.542	5.554
Mean	5.504	5.532	5.506	5.485	5.484	5.511	5.478	5.468	5.496

Grand mean = 5.496
Coefficient of variation = 0.94%
LSD at 0.05 alpha level for location means = 0.052

not statistically different than the value for College Station, Lewiston, and Headland, was lower than the values obtained for Gainsville, Perkins, and Suffolk.

NPCF for Arachin and Conarachin. The NPCF for both arachin and conarachin also varied with genotypes. The values ranged from 5.271 to 5.563 for arachin and from 5.017 to 5.496 for conarachin. For all the genotypes, except 205, the values of NPCF for arachin were higher than those for conarachin. The mean values of NPCF for arachin and conarachin were 5.455 and 5.220, respectively, and CVs were 1.91 and 3.12%, respectively (Table 3).

The results indicated that the NCPF for peanut is likely to vary from sample to sample and is significantly affected by the crop location. Similar variation also was reported by Mossé (1990) for several cereals, legumes, and oilseeds. He also showed that the true NCPF for rice grains may range from 5.1 to 6.0, while for soybean, sorghum, and barley the values were relatively constant.

One of the plausible explanations for not obtaining a fixed value of NPCF for peanut may be the variation in the relative abundance of arachin and conarachin. These variations may be due to genotype, environment, soil fertility, maturity at harvest, etc. Moreover, arachin and conarachin individually are not homogeneous proteins, as indicated by polyacryamide gel electrophoresis of these proteins. Arachin, which occurs as a monomer or dimer, is comprised of at least three polypeptide chains (Yamada *et al.*, 1979). These polypetide chains may have different nitrogen contents, as has been shown in case of wheat (Graham-Janet, 1963; Nimmo *et al.*, 1963). The changes in the relative abundance and composition of the ethanol soluble or nonprotein nitrogen (NPN), which comprises about 6% of the total nitrogen of peanut kernels (Tharanathan *et al.*, 1975), also may have a bearing on the value of NPCF. This study has illustrated the inherent shortcoming in methodology used to determine protein content on the basis of nitrogen content of the kernels. These methods have been employed routinely by the peanut researchers for comparing samples emanating from breeding, agronomic, and other trials and new estimates are needed to give more accurate data.

Table 3. Effect of genotype and location on NPCF for arachin and conarachin (calculated on the basis of amino acid analysis published by Dawson and McIntosh, 1973).

Genotype	Location	Arachin	Conarachin
Starr	Oklahama	5.468	5.288
Early runner	Georgia	5.466	5.297
Samaru	Nigeria	5.533	5.158
B719	Nigeria	5.545	5.087
Starr	Georgia	5.271	5.076
205	Nigeria	5.329	5.496
Florigiant	Virginia	5.467	5.017
Florigiant	Georgia	5.563	5.344
Mean		5.455	5.220
CV (%)		1.91	3.12
SE		0.037	0.058

Conclusions

The NPCF for peanut varies from sample to sample. The true value of NPCF for a given sample may lie between 5.3 and 5.7. This range, however, may prove to be even wider if data on the amino acid analysis of a greater number of samples are used for calculating NPCF, or if a wider range in genotypes was analyzed because most genotypes in this study were spanish types. Thus, the protein content obtained by using a fixed value 5.46 of NPCF would have a precision of ± 0.7% for the samples having 3.5% N (approx. 18% protein); and, with an increase in nitrogen content, the precision would decrease to ± 1.0% for the samples having 5.5% N (approx. 30% protein). This implies that the protein content thus calculated would be different only when there is a difference of at least 1.4% in the calculated values. Likewise, the actual protein content of two samples could be different even if

the values calculated by using a fixed NPCF are equal. Use of a universal factor of 6.25 (FAO, 1970) for peanut will give estimated protein values much higher than the actual values. Systematic research will be required to understand the relationship between the nitrogen content of peanut kernels and the NPCF.

Literature cited

Amaya, F.J., C.T. Young, and R.O. Hammons. 1977. Tryptophan content of US commercial and South American wild genotypes of genus *Arachis*, a survey. Oléagineux 32:225-229.

AOAC. 1984. Official Methods of Analysis of Association of Official Analytical Chemists, 14th Ed. Assoc. Official Analytical Chemists, Arlington, VA, p 259.

Baker, D. 1982. Report on cereal foods, general referee reports. J. Assoc. Off. Anal. Chem. 65:333-334.

Dawson, R., and A.D. McIntosh. 1973. Varietal and environmental differences in the proteins of the groundnut (*Arachis hypogaea*). J. Sci. Fd. Agric. 24:597-609.

FAO. 1970. Amino acid content of foods. Nutr. Stud. No. 24, FAO, Rome.

Graham-Janet, S.D. 1963. Starch gel electrophoresis of wheat flour proteins. Aust. J. Biol. Sci. 16:342-349.

Hovis, A.R., C.T. Young, and P.Y.P. Tai. 1982. Variation in total amino acid percentage in different portions of peanut cotyledon. Peanut Sci 19:44-46.

Jones, D.B. 1931. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. U.S. Dept. Agr. Circ. No. 183, Washington, DC.

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 seed protein content. J. Agric. Food Chem. 38:18-24.

Nimmo, C.C., M.T. O'Sulivan, A. Mohammad, and J.W. Pence. 1963. Fractional and zone electrophoresis of proteins of water-soluble material of flour. Cereal Chem. 40:390-398.

Sosulski, F.W., and G.I. Imafidon. 1990. Amino acid composition and nitrogen to protein conversion factors for animal and plant foods. J. Agric. Food Chem. 38:1351-1356.

Tharanathan, R.N., D.B. Wankehede, and M.R.R. Rao. 1975. Carbohydrate composition of groundnut. J. Sci. Fd. Agric. 26:749-754.

Tkachuk, R. 1969. Nitrogen to protein conversion factors for cereals and oilseed meals. Cereal Chem. 46:419-423.

Yamada, T., S. Aibara, and Y. Morita. 1979. Dissociation-association behaviour of arachin between dimeric and monomeric forms. Agric. Biol. Chem. 43:2549-2556.

Young, C.T. 1979. Amino acid composition of peanut (*Arachis hypogaea* L.) samples from the 1973 and 1974 uniform peanut performance tests. Proc. Amer. Peanut Res. Educ. Soc. 11:24-42.