

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 to Seed Protein Content

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The main two kinds of usual nitrogen to protein conversion factors (k_A and k_P) were investigated from the amino acid composition of seed samples with widely varying N content for 10 cereal species and for several legumes and oilseeds. The variations of these factors as a function of seed N content were determined, and their values were compared with the few data from the literature. It was shown that these two factors are the upper and lower limits, respectively, of the total seed N to true protein conversion factor k , which is close to the average of k_A and k_P . This enabled determination of the factor k with an improved reliability and also comparisons of k values either within a species for distant seed N contents or for a given N content between different species. The conversion factor k varied from 5.1 for N-poor rice grains to 6.0 for N-rich foxtail millet grains. On an average per species, all other species ranged between those two extreme values. They are lower than generally acknowledged until now.

Today, cereal grains and legumes or oilseeds remain by far the predominant source of protein used for human food and for farm animal feed. It is thus important to have an accurate knowledge of the protein concentration of grains. This becomes more and more indispensable in many areas: in human or animal nutrition, in marketing grain according to protein content, in the feed industry, in practical feeding of farm animals (mainly the monogastric ones), and particularly in scientific experimental research requiring definite rations. Recent advances in animal nutrition have revealed that excess, as well as lack of, protein (or of a few particular essential amino acids (AAs) that change according to the animal species involved) can be detrimental. For instance, not only is any excess of protein uneconomical and costly but it is also harmful for environment due to the problem raised by feces elimination of monogastric animals.

The protein concentration of grain is obtained by multiplying its total nitrogen (N) content by a nitrogen to protein (N:P) conversion factor calculated from the AA composition of grain. In other words, the (true) N:P conversion factor k can be defined as the ratio of total protein content to total N content in grain (or in any other biological product). On a theoretical basis this definition seems clear and simple. However, in fact, the analytical recovery of AA analyses cannot reach 100% and N can be evaluated by different ways. Therefore, as discussed in the present paper, there are several ways to assess the N:P conversion factor. Moreover, the values used for this factor by many authors, including FAO (1970), WHO (1973), and AOAC recommendations (Baker, 1979), are partially conventional and frequently erroneous because they are based on inaccurate data and on forgotten assumptions. Otherwise, they are often mistaken for the inverse of the N percentage of total proteins. Though this latter choice often coincides with the use of improved AA data, it remains erroneous, due to the occurrence of non-protein N (NPN). Moreover, the far most prevailing way to express AA composition, even in nonnutritional research, is as grams of AA per 16 g of total N: This indirectly implies that the conversion factor is $100/16 = 6.25$, a

value never valid in plant material, as it has been successively emphasized by Jones (1931), Heathcote (1950), Tkachuk (1966b, 1969, 1977), Heidelbaugh et al. (1975), Sosulski and Holt (1980), de Rham (1982), and Smith (1987).

As early as 1896, for wheat grain, Teller (1932) selected the factor 5.7, which is less inaccurate than that (5.83) calculated by Jones (1926). Jones (1931) extended his calculation to other species. He formulated clearly the problem, so that the questionable values he suggested remain still widespread today, in spite of various improvements successively made by Heathcote (1950), Kutscher and Langnau (1965), Tkachuk (1966a,b, 1969, 1977), Tkachuk and Irvine (1969), Ewart (1967), Holt and Sosulski (1979), Sosulski and Holt (1980), and Morr (1981, 1982). Most of these authors determined the factors from more valid AA data that became available during the 1960s. The most accurate AA compositions ever published until recent years are those of Tkachuk and Irvine (1969) who determined AAs from five different hydrolysates per sample and those of Sosulski and Holt (1980). It is strange that neither the FAO (1970) nor the WHO (1973), in their data or recommendations, used the results of Tkachuk (1969), which correspond to real progress. The same is true for the AOAC (1980) who has been "seeking comments and supporting data on N:P conversion factors".

However, Tkachuk and Irvine (1969) and Tkachuk (1969) analyzed only one single (or two) composite sample(s) per species, so that their results "could not be used to indicate differences in AA composition as influenced by variety or protein content". This latter kind of research has been undertaken during the last years in our laboratory for wheat, maize, pea, lupin, triticale, sorghum, rice, and foxtail millet (Mossé et al., 1985; Baudet et al., 1986a; Mossé et al., 1986, 1987a,b, 1988a-c, 1989), for rye (Baudet et al., 1987), for barley (Huet et al., 1988), and also for oats, pearl millet, French bean, broad bean, soybean, and sunflower seeds (unpublished results). For most of these 16 species, the conditions of AA analysis were generally similar to those used by Tkachuk and Irvine (1969), with six different hydrolysates per seed sample. More-

Table I. Description of Seed Analyzed and Conditions Utilized for Amino Acid Determination

species	samples	cv.	samples/cv. (max) ^a	% N in seed dm (N)			duration or purposes of hydrolyses					ref ^c	
				low	high	range	15 h	24 h	48 h	18 h ^b	Trp		amide
wheat	30	12	11	1.42	3.27	1.85							1
triticale	19	7	11	1.70	3.06	1.36							2
barley	9	7	3	1.45	4.01	2.56							3
maize	30	13	5	1.03	2.95	1.92							4
sorghum	12	7	6	1.50	2.97	1.47	×	×	×	×	×	×	5
rice	8	8	1	1.27	2.07	0.80							6
pea	33	11	14	2.84	5.15	2.31							7
lupin	20	10	6	3.80	7.75	3.95							8
soybean	6	3	3	4.90	6.94	2.04							9
pearl millet	20	20	1	1.10	3.34	2.24							9
foxtail millet	13	7	2	1.82	3.65	1.83		×	×	×	×		10
oats	56	21	17	1.02	3.80	2.78							9
broad bean	24	24	1	3.73	6.04	2.31		×		×	×		9
sunflower	7	7	1	2.61	5.39	2.78		×	×	×			9
French bean	5	5	1	3.39	4.89	1.50		×		×			9
rye	13	10	4	1.29	4.37	3.08		×			×		11

^a Highest number of samples per cultivar (cv.). ^b After previous performic oxidation. ^c Key: (1) Mossé et al. (1985), (2) Mossé et al. (1988a), (3) Huet et al. (1988), (4) Baudet et al. (1986a), (5) Mossé et al. (1988b), (6) Mossé et al. (1988c), (7) Huet et al. (1987), (8) Mossé et al. (1987b), (9) unpublished results, (10) Mossé et al. (1989), (11) Baudet et al. (1987).

over, on an average, about 20 samples per species were selected from a number of varieties and for their wide range of protein content.

The purpose of the present paper was to show that, in the absence of perfectly accurate values of the conversion factor, it is still possible to accurately determine its upper and lower limits, which are often close to each other; to describe quantitatively the variations of these limits as a function of grain N content; and within these limits, to assess improved values of this factor with a known degree of uncertainty.

MATERIALS AND METHODS

Materials. The main characteristics of the materials analyzed are reported in Table I. They comprise seeds from 16 species: 10 cereals, 5 legume seeds (including soybean), and 1 oilseed. The number of samples analyzed varied from 5 for soybean to 33 for pea seeds. For several species about one-third to half of the samples studied belonged to the same cultivar (or variety, inbred line, or hybrid according to the species), in order to investigate separately the influence of environment on AA composition (i.e., phenotypic variation) while the other samples were selected from different cultivars in order to study the possible influence of genome. For each species, the seed N percentage on a dry basis (N) spread over the widest possible range (Table I).

Analytical Methods. Seed sampling, milling, meal subsampling, meal dry matter, and total N determinations and AA analyses were performed under conditions previously detailed elsewhere (Mossé et al., 1985). For seven of the species investigated, amide ammonium was not determined and for two of them tryptophan was not analyzed, while the other AAs were determined from two or three hydrolysates only. However, for the nine other species AAs were determined from six different hydrolysates. In order to make allowance for losses resulting either from partial degradation or from incomplete release, three hydrolyses (15, 24, 48 h) were performed in boiling 6 M HCl in addition to an 18-h hydrolysis of a previously oxidized sample for sulfur AAs. Amide NH₃ from asparagine plus glutamine was determined after a separate hydrolysis (3 h in 2 M HCl at 115 °C) and tryptophan from an alkaline hydrolysate in Ba(OH)₂.

It must be emphasized that in addition to the utilization of nonbiological AAs such as norleucine or α -amino- β -guanidopropionic acid, which were added to the mixture of common AAs (i.e., protein AAs) for calibration of the analyses, aliquots of a long-term stored hydrolysate of a laboratory-defatted soybean meal were used as standards for each ninhydrin preparation to monitor the reproducibility. Moreover, commercial egg white lysozyme (Merck) and purified human serum albumin (NBC)

were used as reference proteins to check the accuracy of AA determinations. Thus, AA compositions used here probably represent the most complete analyses of the total proteins of cultivated seeds.

Definition and Calculation of the Conversion Factor.

It has been previously emphasized (Mossé et al., 1985) that the conversion factor can be defined in several ways. A first definition corresponds to the ratio

$$k_A = \sum E_i / \sum D_i \quad (1)$$

of actual seed proteins (that is total AA residue weight) to total N recovered from the 20 AAs (amide N of asparagine and glutamine being included). The calculation of k_A necessitates the use of two expressions of AA composition of seeds: (1) as grams of residue (E_i) of the i th AA/100 g of seed dry matter (a residue is an anhydrous AA; i.e., the actual molecular fraction of an AA bound and copolymerized in polypeptide chains); (2) as grams of N (D_i) of the i th AA/100 g of seed dry matter. The total weight of residues $\sum E_i$ includes the 20 common protein AAs. Although data are always restricted, at the best, to 18 figures plus the amide NH₃, the total of glutamic acid plus glutamine Glx = Glu + Gln is always determined because glutamine gives glutamic acid during acid hydrolysis by substitution of a carboxyl group (COOH) to its amide group (CONH₂). By chance, the MW values of Glu (147.13) and Gln (146.15) are close enough to describe their total Glx in terms of Glu. The same is true for the total of aspartic acid and asparagine (MW 133.11 and 132.12, respectively): Asx = Asp + Asn. It is obvious that neither the amide NH₃ nor the analytical NH₃, which can be determined in seed meal hydrolysates, has to be included in the total $\sum E_i$, although some authors do so erroneously. However, the total weight $\sum D_i$ of AA N includes the 19th figure: that is, the amide N released as NH₃ by asparagine and glutamine when they are converted by acid hydrolysis into glutamic and aspartic acids. For lack of specific amide N determination, the total NH₃ determined from 6 M HCl hydrolysates can be used (Heathcote, 1950; Tkachuk, 1969) by keeping in mind that this total NH₃ amount is generally equal to, or sometimes slightly higher than, amide NH₃. In order to be as rigorous as possible, it is preferable to distinguish a factor k_A' (close to k_A) when amide NH₃ is replaced by hydrolysis NH₃ in the total $\sum D_i$.

Another useful factor corresponds to the ratio

$$k_p = \sum E_i / N \quad (2)$$

of actual seed proteins to total seed N content in 100 g of seed dry matter (N being determined by the micro-Kjeldahl method).

Another factor, k_N , giving approximately the weight of the main N compounds (proteins plus nucleic acids) from N has

Table II. Slopes (u or p , \pm SD), Intercepts (v or q , \pm SD), and Correlation Coefficients r of Regression Lines Representing Total Amino Acid Content as a Function of Nitrogen Content (N), as Grams of Amino Acid Residues $\sum E_i = uN + v$, or as Grams of Amino Acid N $\sum D_i = pN + q$, Respectively, in 100 g of Seed Dry Matter

species	$u \pm$ SD	$v \pm$ SD	r	$p \pm$ SD	$q \pm$ SD	r	ref
wheat	5590 ^a \pm 59	-670 \pm 150	998	989 \pm 10	-72 \pm 25	999	1 ^b
triticale	5375 \pm 115	-422 \pm 261	996	959 \pm 16	-78 \pm 36	998	2
rye ^c	5174 \pm 176	-143 \pm 511	994	292 \pm 23	-30 \pm 66	997	11
barley	5195 \pm 81	130 \pm 206	999	904 \pm 12	30 \pm 31	999	3
corn	5880 \pm 50	-720 \pm 90	999	1001 \pm 8	-100 \pm 13	999	4
sorghum	5550 \pm 112	-87 \pm 245	998	955 \pm 21	-14 \pm 45	998	5
pearl millet ^c	5159 \pm 69	21 \pm 154	998	917 \pm 11	41 \pm 26	999	9
foxtail millet ^c	6372 \pm 208	-1381 \pm 524	994	1055 \pm 37	-158 \pm 93	993	10
rice	5650 \pm 167	-748 \pm 271	997	1020 \pm 20	-136 \pm 33	999	6
oats ^c	5550 \pm 128	-767 \pm 329	990	1005 \pm 18	-137 \pm 46	994	9
pea	5122 \pm 66	583 \pm 260	997	1003 \pm 12	-204 \pm 49	998	7
lupin	5253 \pm 61	128 \pm 360	999	1030 \pm 14	-392 \pm 85	998	8
soybean	5552 \pm 224	-1072 \pm 1257	997	1024 \pm 41	-469 \pm 232	997	9
broad bean	5366	-1024					9
French bean	4789	1404					9
sunflower	5142	-435					9

^a All the figures of the table have been multiplied by 10³. ^b See Table I, footnote c. ^c Species for which total AA nitrogen was determined by taking into account the NH₃ eluted with AAs instead of that resulting from amide groups only.

theoretical interest only and was not considered in the present paper. As for the accurate factor k allowing determination of the accurate content of actual seed proteins from N , it can only be approached with an improved accuracy in a way suggested at the end of this paper.

RESULTS AND DISCUSSION

Variation of the Conversion Factors as a Function of Seed Nitrogen Content. In recent papers dealing with AA composition of 10 cereal and legume species and listed in Table I, we have shown that the level of each common AA (i.e., each of the 20 AAs that commonly occur in protein molecules) in seed dry matter increases linearly with N . It follows that their total contents $\sum E_i$ (as grams of AA residues/100 g of seed dry matter) and $\sum D_i$ (as grams of AA N/100 g of seed dry matter) also increase as linear functions of N described by

$$\sum E_i = uN + v \quad (3)$$

$$\sum D_i = pN + q \quad (4)$$

in which the coefficients u , v , p , and q are constant within a species. The values of these coefficients are reported in Table II with corresponding standard deviations (SD) and correlation coefficients r . It is worth noting that r values are close to 1 and all are higher than 0.99, which corresponds to a significance level equal to 10⁻⁴ or 10⁻⁵ for most of them. This allows assessment of the conversion factors defined above:

$$k_A = (uN + v)/(pN + q) \quad (5)$$

$$k_P = u + v/N \quad (6)$$

This shows that both factors change as quadratic functions of N represented by segments of equilateral hyperbola. According to the species and to the kind of factor, the latter can either increase or decrease as a function of N , that is, as a first approximation, as a function of total seed protein content. As examples, the variations of k_A and k_P for corn and pea are drawn in Figure 1. This figure shows that for pea both kinds of factors decrease as a function of N . The same is true for lupin seed (Mossé et al., 1987), while k_P slightly increases contrary to k_A for soybean. For all cereals except barley, k_P increases, whereas k_A can either remain constant or increase according to the species. Equations 5 and 6 and data reported

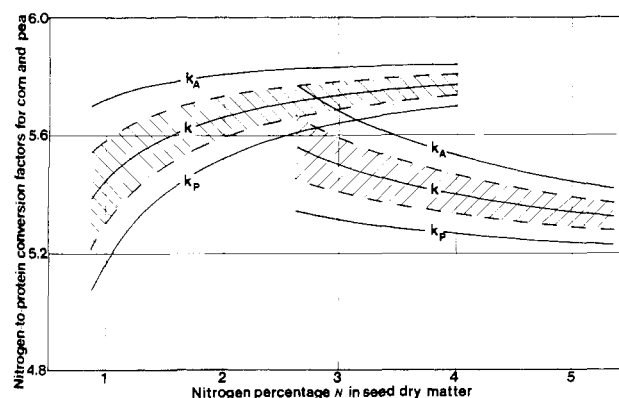


Figure 1. Variation in the N to protein conversion factors k_A , k_P , and k as a function of seed N percentage N (on a dry matter basis) for corn (left side) and pea (right side). The plausible deviation for k is represented by the striped area.

in Table II also show that, for all the species, the variations of both factors are always more significant at low, than at high, N values. Figure 1 suggests that for a given N value k_A is higher than k_P , which is obvious. Since $\sum D_i$ corresponds to total AA N only, while N corresponds to total (AA plus nonprotein) seed N, it follows that $\sum D_i < N$. As a consequence, $\sum E_i/\sum D_i > \sum E_i/N$. That is

$$k_A > k_P \quad (7)$$

It can also be noted that the N recovery (R) in AA analyses, which equals the ratio of AA N to total seed N ($R = \sum D_i/N$) is always < 1 . According to eq 1 and 2

$$k_P = Rk_A \quad (8)$$

which means that k_P is lower than k_A .

Comparison with Literature Data. Although it is devoid of difficulty, calculation of the conversion factors is sophisticated enough to result in some miscalculation. The most frequent but minor error in the literature consists of taking into account the weight of amide (or analytical) NH₃ in the calculation of total weight ($\sum E_i$) of AA residues, as did Heathcote (1950) and Tkachuk (1966a, 1977) who were the first to improve the method of conversion factor determination by using AA compositions, as did Morr (1981). Another kind of error results from confusion between total weight of AAs and total weight of their residues. Therefore, the values of k_A' deter-

Table III. Comparison between Literature Values^a (LV) and Present Work (PW) for N Recovery *R* from AA Analysis and for Conversion Factors k_A and k_P

species	<i>N</i> ^b	% <i>R</i>		k_P			k_A		
		LV	PW	LV	PW	% Δ ^c	LV	PW	% Δ ^c
wheat	2.94	95.8	96.4	5.37	5.36	0.2	5.61	5.56	0.9
	2.86	96.3	96.4	5.40	5.36	0.7	5.61	5.56	0.9
triticale	3.15	95.3	93.4	5.49	5.24	4.8	5.76	5.61	2.7
rye	2.53	89.6	91.7	5.05	5.12	-1.4	5.63	5.58	0.9
barley	2.14	90.5	91.8	5.13	5.26	-2.5	5.66	5.72	-1.0
pearl millet	2.37	98.4	93.4	5.59	5.17	8.1	5.68	5.53	2.7
oats	3.13	92.7	96.1	5.10	5.31	-3.6	5.50	5.52	-0.4
pea	4.57	95.1	95.8	5.25	5.25	0	5.52	5.48	-1.0
lupin	6.17	92.9	96.6	4.94	5.27	-6.3	5.32	5.46	-2.6
soybean	6.18	92.8	94.8	5.22	5.38	-3.0	5.63	5.67	-0.7
broad bean	4.47	91.4	n.d.	5.03	5.13	-2.0	5.50		
French bean	4.61	89.4	n.d.	5.11	5.09	0.4	5.71		

^a According to, or calculated from, data of Tkachuk (1969) for cereals and Sosulski and Holt (1980) for legumes. ^b Seed nitrogen percentage on a dry basis. ^c Relative difference between present work and literature values expressed as the percentage $100(LV - PW)/PW$.

mined in a first step by Morr (1981) for several soybean protein products have been ca. 18% overestimated. Afterward, they were correctly recalculated (Morr, 1982). The data reported in Table II show that for soybean k_A decreases from 5.78 to 5.63 when *N* increases from 4.5 to 7.3. These values agree with those of Morr (1982) as well as with those that can be worked out from AA compositions published by Smith and Circle (1972): All these determinations of k_A' for selected soy protein products actually range between 5.63 and 5.80.

The values of k_A (or k_A') and k_P obtained from Table II for a given *N* can be compared with published data or calculated from data available in the literature. Such a comparison is shown in Table III for cereals investigated by Tkachuk and Irvine (1969) and Tkachuk (1969) and for legume seeds analyzed by Holt and Sosulski (1979) and Sosulski and Holt (1980). In most cases the agreement is striking. For k_A (or k_A') the relative deviations between our results and those from these authors are less than, or close to, 1%. The agreement is also good for k_P of which determination depends on *R*, as discussed later on. The relative deviations are greater than $\pm 5\%$ for pearl millet and for lupin only. They can be easily explained. Although the millet sample has been analyzed with great care by Irvine and Tkachuk (1979), the N recovery (*R*) of this sample is still abnormally high: 98.4%, instead of 93.4% in the present work, while that of the average of the two lupin samples is low (92.9% instead of 96.6%). This plausibly results from an imperfect calibration in AA determination leading to some overestimation (or underestimation for lupin) of total weight $\sum E_i$ of residues. For legume seeds, Sosulski and Holt (1980) performed only one 24-h acid hydrolysis (instead of three) plus particular hydrolyses for S AAs, tryptophan, and amide N. For broad bean, pea, and French bean they published average AA compositions of 6, 17, and 4 samples, respectively, which plausibly explains the reliability of their results. They analyzed only two lupin seed samples: This might be the cause of discrepancies with the present results. The same is true for Ewart (1967) who determined k_A in single samples of five cereal species after hydrolysis conditions close to those of Sosulski and Holt (1986). His results (not reported in Table III) are $2.5 \pm 1\%$ higher than those of the present work.

Significance of the Factors k_A and k_P . It is worth noting that k_A obtained from AA composition is a N:P conversion factor—and the true one—for a pure protein only or for mixtures in which there are no other N compounds than proteins. Its reverse, $100/k_A$, provides the N percentage in the protein(s) concerned. From a theoretical standpoint, this percent N varies from 8.58 for

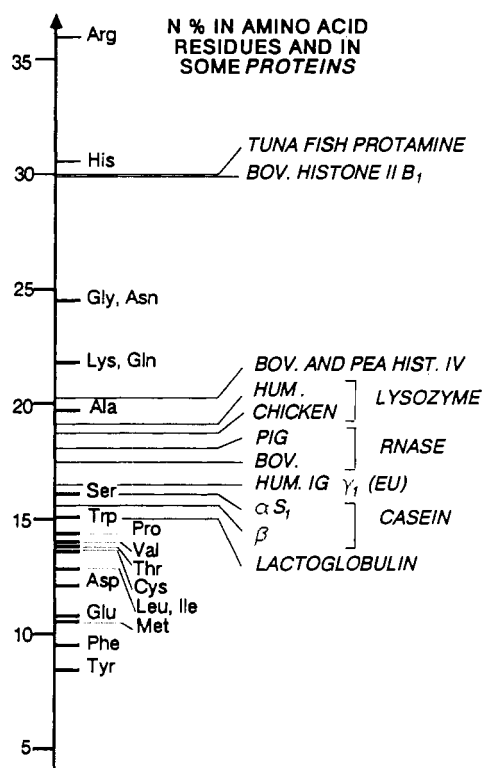


Figure 2. Percent N in the common AA residues and in a few proteins. Key: Bov = bovine; Hum = human.

polytyrosine to 35.87 for polyarginine. Its values in common AA residues are displayed in Figure 2 with those ones of some proteins. The latter were selected from those of which the AA sequence is known (Dayhoff, 1972), that is the proteins in which accurate amounts of each of the four AAs have been determined: asparagine, aspartic acid, glutamine, and glutamic acid. This figure shows that AAs are distributed in three groups according to percent N in their residues. Tyrosine, phenylalanine, methionine, and glutamic acid residues are N poor (from 8.58 to 10.85%) while lysine, glutamine, asparagine, glycine, histidine, and arginine residues are N rich (from 21.86 to 35.87%). For the other 10 AA residues, percent N ranges within $16 \pm 4\%$ (from 12.17% for aspartic acid to 19.7% for alanine). It is thus unlikely that percent N in a pure protein, and all the more so in a protein mixture, be less than 15% or more than 30%.

According to eq 1 this percent N in total seed proteins equals $100\sum D_i/\sum E_i$. Therefore, its variations as a function of total seed *N* are represented by segments of equi-

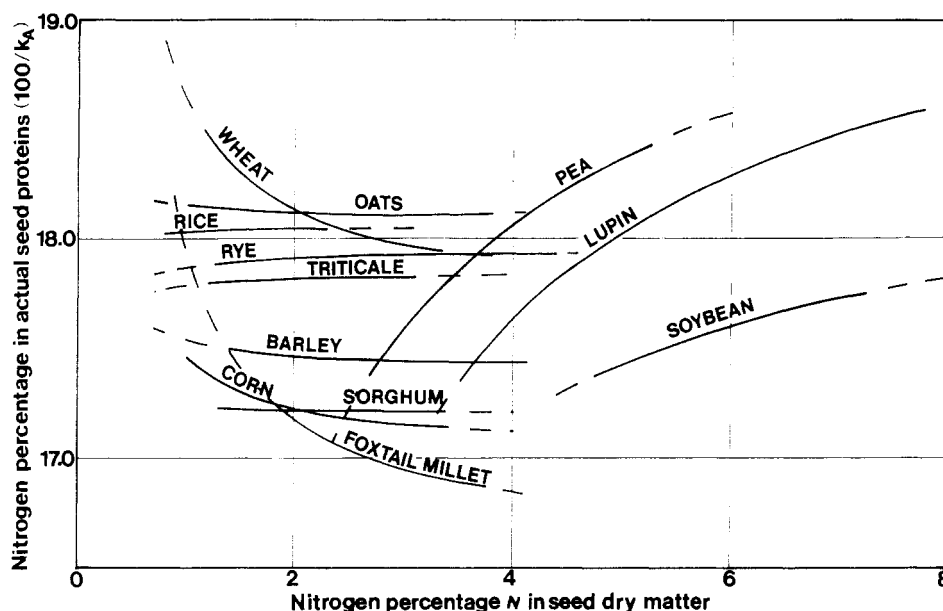


Figure 3. Variation in the percent N in total and actual seed proteins as a function of seed N percentage N for several cereal and legumes.

lateral hyperbola drawn in Figure 3. It significantly decreases as a function of N for wheat, corn, and particularly foxtail millet. It is virtually constant for barley, triticale, rye, rice, and oats, whereas it more or less significantly increases for the three legume seeds represented. In any case, it remains within the range $17.9 \pm 1\%$.

For total storage proteins accumulated in different amounts in seeds according to N (Mossé et al., 1986, 1988b, 1989; Huet et al., 1987), it is possible to calculate their percent N equal to $100p/u$. Surprisingly, despite the fact that they consist of mixtures of numerous polypeptide chains (plausibly more than 100 or so for cereal grains), the percent N of seed storage proteins differs significantly according to the species and ranges from 16.6% for foxtail millet to 19.6% for pea and lupin.

An advantage of k_A (or of its reverse) determination is that, according to eq 1, it is practically independent of N recovery in AA analyses, provided that R does not have an abnormal value. In fact, R is the product of two terms, R_1 and R_2 , each of which is lower than unity. R_1 is the actual analytical recovery of AAs expressed here as percent N. Due to slight alterations or losses of minute amounts of AAs, mainly during hydrolysis, $R_1 < 1$. However, such losses result in a similar decrease of $\sum E_i$ and $\sum D_i$ without significant changes in their ratio k_A . This can still be a little less valuable for k_A' for which analytical NH_3 is used instead of amide NH_3 . As for R_2 , it is the ratio of total common AA N to total seed N, and $1 - R_2$ indicates the NPN amount. Outside of mixtures of pure proteins (where $R_2 = 1$), $R_2 < 1$. This distinction between R_1 and R_2 has been neglected until now in the careful studies of both Holt and Sosulski (1979) and Sosulski and Holt (1980) as well as by myself in the papers cited in Table I. Though R_1 and R_2 are probably close to each other, their accurate determination is practically impossible. Several attempts have tried to determine R_2 , but they rely on extraction techniques that cannot be quantitative enough and only give an approximate magnitude (Holt and Sosulski, 1981; Baudet et al., 1986a). As suggested by Teller (1932), Heathcote (1950), Tkachuk (1969, 1977), and Sosulski and Holt (1980), k_A must be corrected because it does not make allowance for NPN. Therefore, it does not allow calculation of the real pro-

tein amount from total seed N . However, in any case, it must be emphasized that k_A is a little higher than the upper limit of the true conversion factor, $k < k_A$.

On the other hand, outside industrial food protein products, k_A has little use in practice. For instance, the present work shows that k_A is close to 5.7 for soybean proteins and this value is the real conversion factor for purified soy protein isolates.

k_P has been named the "corrected conversion factor" by Sosulski and Holt (1980) who are the first to have published values of both kinds of conversion factors. k_P has indeed the advantage of taking into account NPN. This is a reason explaining eq 7 and results from the fact that $R_2 < 1$. However, eq 7 also results from the fact that k_P depends on analytical recovery ($R_1 < 1$) of AAs during their analysis. Indirect evidence shows that both R_1 and R_2 slowly increase with N in most species (unpublished results). As a consequence, the true conversion factor k is a little higher than k_P , which corresponds to the lowest limit that may be reached by k (if R_1 could equal 100%). In other words, k is always within a range narrower than that limited by k_P and k_A :

$$k_A < k < k_P \quad (9)$$

Assessment of the True N:P Conversion Factor k . Calculation from data reported in Table II shows that, according to the species investigated, the relative difference $100(k_A - k_P)/0.5(k_A + k_P)$ generally decreases as a function of N and ranges between 6 and 10% at low N and between 1.5 and 4% at high N of the species concerned. This leads to the assumption that k is very close to the average of k_A and k_P with possible deviations that plausibly do not exceed $\pm(k_A - k_P)/4$ as it is represented for corn and pea in Figure 1. Such an assumption allows quantification of k as a function of N by

$$k = (k_A + k_P)/2 \pm (k_A - k_P)/4 \quad (10)$$

The variations of k as a function of N according to such a definition are represented in Figure 4 for the cereals and legumes investigated here. For rye, pearl millet (not shown because its k variations are close to those of rye), barley, soybean, and sorghum, k can be considered as constant and virtually independent of N . For pea and lupin it decreases about 0.1 unit within the N range of

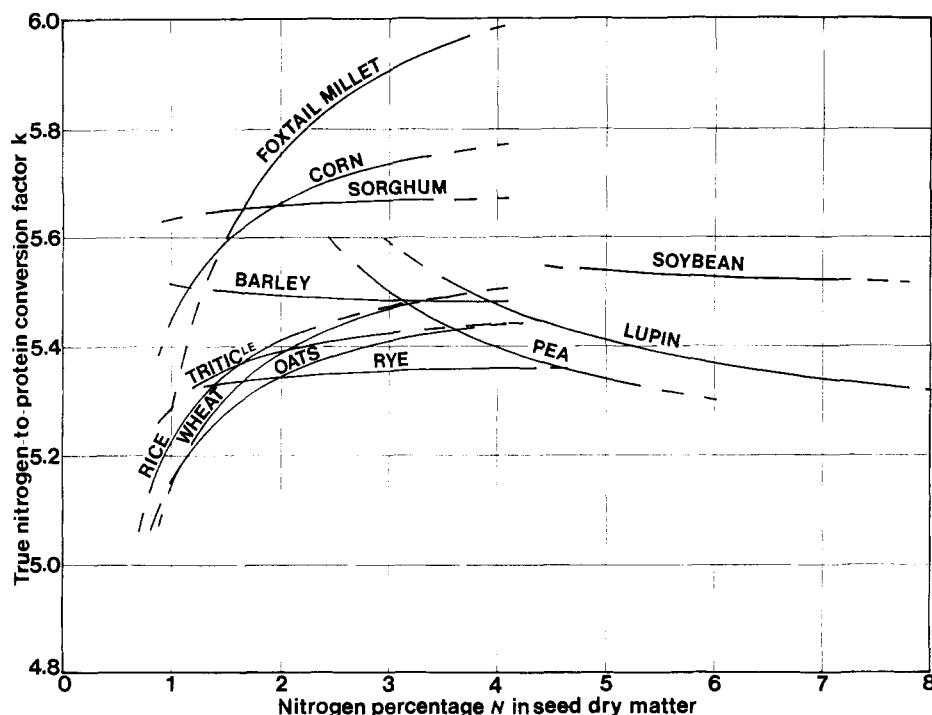


Figure 4. Variation in the N to protein conversion factor k as a function of N for several cereal and legumes.

these seeds. On the contrary, for wheat, corn, foxtail millet, oats, and rice, it significantly increases with N . Its highest variations occur in foxtail millet for which it increases from 5.28 for $N = 1\%$ to 5.98 for $N = 4\%$. The present results also show that for cereals k variations from $N = 1$ to 2% are roughly twice as much as they are from $N = 2$ to 4% . Since, in developed countries, the most frequent N value is ≥ 2 for many cereals, it can appear useless to take into account data on the variation of k according to N . However, for rice, the most frequent N value is around 1.2 (for many Asian rice samples N ranges from 0.8 to 1.5%) and this variation is really significant. On the other hand, it must be kept in mind that N is determined on aliquots of tremendous numbers of grains. For instance, on the world scale according to FAO (1988), the average yield of wheat is now higher than 2 metric tons/ha (i.e., per 2.5 acres), that is more than 50 million wheat grains/ha. Therefore, N is an average value of widely N ranged grains. As new processes for screening grains according to N on silo scale are going to emerge in the future, this may render knowledge of k variation according to N useful.

A simplified way still consists of the use of averaged values of k for the species investigated. These approximate values are, in decreasing order as follows: foxtail millet, 5.8 ± 0.2 ; corn, 5.65 ± 0.15 ; sorghum, 5.65 ± 0.02 ; soybean, 5.52 ± 0.02 ; barley, 5.50 ± 0.02 ; pea, 5.44 ± 0.14 ; lupin, 5.40 ± 0.08 ; triticale and oats, 5.36 ± 0.05 ; wheat, 5.33 ± 0.17 ; pearl millet, 5.33 ± 0.05 ; rye, 5.33 ± 0.03 ; rice, 5.17 ± 0.25 . From this viewpoint, it is possible to calculate k for other legumes and for single samples (corresponding to single N values) of oilseeds analyzed by Sosulski and Holt (1980) and Tkachuk (1969). Calculation gives $k = 5.40$ for broad bean, 5.33 for French bean, and 5.30 for rapeseed and sunflower samples (with N close to 5% in nondefatted oilseeds). The present results show that k varies from 5.13 for N-poor rice samples to about 6.0 for N-rich foxtail millet samples. It is thus true that substantial differences can reach 20% in protein content for grains corresponding to equal N as noted by Heidelbaugh et al. (1975) when these authors inves-

tigated the food system involved in the skylab manned space flight program.

On an average between species, the (true) conversion factor k is often close to 5.4 or 5.5, rather than 5.7 or 6.25, as generally admitted. However, as compensation, the lower k is, the higher the AA content in seed proteins. As an example, with the conventional factor 6.25 (which implies that 100 g of protein correspond to $100/6.25 = 16$ g of N) for $N = 2\%$, lysine content has been shown to equal 2.6 g/16 g of N for corn and 2.9 for wheat (Baudet et al., 1986; Mossé et al., 1985). The present work shows that these contents equal 2.9 g/100 g of actual proteins for corn and 3.35 for wheat.

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LITERATURE CITED

- AOAC. Nitrogen-to-protein conversion factor. *J. Assoc. Off. Anal. Chem.* 1980, 63, 678.
- Baker, D. Report on Cereal foods. *J. Assoc. Off. Anal. Chem.* 1979, 62, 369-370.
- Baudet, J.; Huet, J.; Jolivet, E.; Lesaint, C.; Mossé, J.; Pernollet, J. C. Changes in accumulation of seed nitrogen compounds in maize under conditions of sulphur deficiency. *Physiol. Plant.* 1986a, 68, 608-614.
- Baudet, J.; Huet, J. C.; Mossé, J. Variability and relationships among amino acids and nitrogen in maize grains. *J. Agric. Food Chem.* 1986b, 34, 365-370.
- Baudet, J.; Huet, J. C.; Mossé, J. Interdépendance entre la composition en acides aminés du grain de seigle et son taux d'azote. *Agronomie* 1987, 7, 813-819.
- Dayhoff, M. O. *Atlas of protein sequences and structure*; National Biomedical Research Foundation: Washington, DC, 1972; Vol. 5.
- Ewart, J. A. D. Amino acid analyses of cereal flour proteins. *J. Sci. Food Agric.* 1967, 18, 548-552.
- FAO. Amino acid content of foods. *Nutr. Stud.* 1970, No. 24.
- FAO. *Yearbook—Production 1987*; FAO Statistics Series 82; FAO: Rome, 1988; Vol. 41, 351 pp.

- Heathcote, J. G. The protein quality of oats. *Br. J. Nutr.* 1950, 4, 145-154.
- Heidelbaugh, N. D.; Huber, C. S.; Bednarczyk, J. F.; Smith, M. C.; Rambaut, P. C.; Wheeler, H. O. Comparison of three methods for calculating protein content of foods. *J. Agric. Food Chem.* 1975, 23, 611-613.
- Holt, N. W.; Sosulski, F. W. Amino acid composition and protein quality of field peas. *Can. J. Plant Sci.* 1979, 59, 653-660.
- Holt, N. W.; Sosulski, F. W. Nonprotein nitrogen contents of some grain legumes. *Can. J. Plant Sci.* 1981, 61, 515-523.
- Huet, J. C.; Baudet, J.; Mossé, J. Constancy of composition of storage proteins deposited in *Pisum sativum* seeds. *Phytochemistry* 1987, 26, 47-50.
- Huet, J. C.; Baudet, J.; Bettaieb Ben Kaab, L.; Mossé, J. Variation of the amino acid scores and the nitrogen to protein conversion factor in barley grain as a function of nitrogen content as compared with wheat and rye. *Plant Foods Hum. Nutr.* 1988, 38, 175-188.
- Jones, D. B. A new factor for converting the percentage of nitrogen in wheat into that of protein. *Cereal Chem.* 1926, 3, 194-198.
- Jones, D. B. Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. *U.S., Dep. Agric., Circ.* 1931, No. 183, 21 pp.
- Kutscher, D. W.; Langnau, D. Über die biologische Wertigkeit von Soja-Eiweiss und aufgeschlossenem Milcheiweiss. Eine kritische Betrachtung. *Fleischwirtschaft* 1965, 45, 1432-1434.
- Morr, C. V. Nitrogen conversion factors for several soybean protein products. *J. Food Sci.* 1981, 46, 1362-1367.
- Morr, C. V. Recalculated nitrogen conversion factors for several soybean protein products. *J. Food Sci.* 1982, 47, 1751-1752.
- Mossé, J.; Huet, J.; Baudet, J. C. The amino acid composition of wheat grain as a function of nitrogen content. *J. Cereal Sci.* 1985, 3, 115-130.
- Mossé, J.; Huet, J. C.; Baudet, J. Evidence from amino acid analysis for the constancy of composition of storage protein accumulated in maize seed. *J. Plant Physiol.* 1986, 125, 199-205.
- Mossé, J.; Huet, J. C.; Baudet, J. Changements de la composition en acides aminés des grains de pois en fonction de leur taux d'azote. *Sci. Aliment.* 1987a, 7, 301-324.
- Mossé, J.; Huet, J. C.; Baudet, J. Relationships between nitrogen, amino acids and storage proteins in *Lupinus albus* seeds. *Phytochemistry* 1987b, 26, 2453-2458.
- Mossé, J.; Huet, J. C.; Baudet, J. The amino acid composition of triticale grain as a function of nitrogen content. Comparison with wheat and rye. *J. Cereal Sci.* 1988a, 7, 49-60.
- Mossé, J.; Huet, J. C.; Baudet, J. The amino acid composition of whole sorghum grain in relation to its nitrogen content. *Cereal Chem.* 1988b, 65, 271-277.
- Mossé, J.; Huet, J. C.; Baudet, J. The amino acid composition of rice grain as a function of nitrogen content as compared with other cereals: a reappraisal of rice chemical scores. *J. Cereal Sci.* 1988c, 8, 165-175.
- Mossé, J.; Huet, J. C.; Baudet, J. Relationships between amino acid composition of nitrogen content of foxtail (Italian) millet grain of different varieties. *J. Sci. Food Agric.* 1989, 46, 383-392.
- Rham, O. de. La proportion d'azote dans les protéines et le facteur de calcul protéine/azote. *Lebensm. Wiss. u. Technol.* 1982, 15, 226-231.
- Smith, D. B. Determination of protein content of seeds in breeding programmes. In *Protein evaluation in cereals and legumes*; Pattakou, V., Ed.; CEC Rep. EUR 1404 EN; CEC: Luxembourg, 1987; pp 21-31.
- Smith, A. K.; Circle, S. J. Chemical composition of the seed. In *Soybeans: chemistry and technology*; Smith, A. K., Circle, S. J., Eds.; AVI: Westport, CT, 1972; pp 61-92.
- Sosulski, F. W.; Holt, N. W. Amino acid composition and nitrogen to protein factors for grain legumes. *Can. J. Plant Sci.* 1980, 60, 1327-1331.
- Teller, G. L. Non-protein nitrogen compounds in cereals and their relation to the nitrogen factor for protein in cereals and bread. *Cereal Chem.* 1932, 9, 261-274.
- Tkachuk, R. Amino acid composition of wheat flours. *Cereal Chem.* 1966a, 43, 207-223.
- Tkachuk, R. Note on the nitrogen-to-protein conversion factor for wheat flour. *Cereal Chem.* 1966b, 43, 223-225.
- Tkachuk, R. Nitrogen-to-protein conversion factors for cereals and oilseed meals. *Cereal Chem.* 1969, 46, 419-423.
- Tkachuk, R. Calculation of the nitrogen-to-protein conversion factor. In *Nutritional standards and methods of evaluation for food legume breeders*; Hulse, J. H., Rachie, K. O., Billingsky, L. W., Eds.; IDRC: Ottawa, 1977; pp 78-82.
- Tkachuk, R.; Irvine, G. N. Amino acid compositions of cereals and oilseed meals. *Cereal Chem.* 1969, 46, 206-218.
- WHO. *Energy and Protein Requirements*; Nutrition Meetings Report Series 52; FAO: Rome, 1973.

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