Nitrogen-to-Protein Conversion Factors for Common Vegetables in Japan

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ABSTRACT: Twenty (JAPANESE) vegetables in Japan were analyzed for total nitrogen, amino acid nitrogen, amide nitrogen, and nonprotein nitrogen and then calculated nitrogen-to-protein conversion factors were calculated. The average conversion factor based on quantitative amino acid composition data was 6.00. However, the use of this factor for estimating protein content led to a considerable difference from estimates based on amino acid residue concentrations, due to the presence of high levels of nonprotein nitrogen. The distribution of protein nitrogen to total nitrogen averaged 73%. Adjusted conversion factors, defined as the proportion of amino acid nitrogen to total nitrogen, averaged 4.39. Protein contents estimated with this factor agreed well with contents determined by amino acid residues.

Key words: nitrate, nitrogen, nonprotein nitrogen, protein, vegetables

Introduction

IT IS CUSTOMARY TO CALCULATE THE amount of total protein in a food by multiplying its Kjeldahl nitrogen content by a nitrogen-to-protein (N:P) conversion factor. Although special conversion factors are used for some foods (Jones 1931), the value of 6.25 is applicable for most plant and animal proteins, based on the assumption that their proteins contain approximately 16% nitrogen. However, the amino acid composition varies from one protein source to another. Also, this assumption is invalid for foods that contain other nitrogenous compounds such as nucleic acids, amines, urea, ammonia, nitrates, nitrites, vitamins, alkaloids, phospholipids, and nitrogenous glycosides. Using the common factor 6.25 for such foods will result in an overestimation of protein content. Therefore, a more practical and reliable method for converting total nitrogen data, as determined by the Kjeldahl method or another appropriate analytical procedure, to measure protein content, taking the effects of nonprotein nitrogen into consideration, needs to be developed.

Many studies have consistently demonstrated that leaf, stem, and flower vegetables are generally rich in nitrate (Hata 1979; Hata and Ogata 1979; Taira and Taira 1979). The amount of total protein in vegetables is calculated by determining the total nitrogen using the Kjeldahl method with the addition of salicylic acid as the catalyst (AOAC 1990) and multiplying this value by 6.25, the traditional conversion factor. Because the Kjeldahl method cannot distinguish between protein nitrogen and nonprotein nitrogen, the protein percentages in vegetables that contain significant nitrate levels may be overestimated.

In a previous paper (Fujihara and others 1995), mushroom varieties common to Japan were selected as model foods for exploring a new method of determining N:P factors and reported that a better N:P conversion factor for these mushrooms was 3.99. Similarly, other nitrogenous compounds, such as nitrate nitrogen and nucleic acid nitrogen, affect the N:P conversion factor for vegetables. Tokoro and others (1987) determined the nitrogen compositions of 12 kinds of vegetables and suggested that the N:P conversion factor for them should be 4.7 instead of 6.25. However, in the above Tokoro's study, the amino acid composition for each vegetable was not estimated. Furthermore, they made reference only to nitrate as nonprotein nitrogen.

The objective of our study was to determine an accurate conversion factor for calculating the protein content of each vegetable from the total nitrogen content, based on amino acid composition and the distribution of nitrogen in protein or other nitrogenous components. In addition, we propose a reliable conversion factor for routinely converting total nitrogen data into results that estimate the protein content of foods is proposed.

Materials and Methods

Materials

Twenty fresh vegetable products were obtained at a local supermarket: cabbage (*Brassica oleracea.* L. *var. capitata.* L.), carrot (*Daucus carota* L.), Chinese cabbage (*Brassica pekinensis* Rupr.), Chinese chive (*Allium tuberosum* Rottl. ex K. Spreng.), cucumber (*Cucumis sativus* L.), edible burdock (*Arctium lappa* L.), eggplant (*Solanum melongena* L.), Japanese radish (*Raphanus sativus* L.), komatsuna (*Brassica rapa* nothovar.), lettuce (*Lactuca* sativa L.), onion (*Allium cepa* L.), pumpkin (*Cucurbita maxima* Duch.), spinach (*Spinacia oleracea* L.), sweet pepper (*Capsicum annuum* L. var. *angulosum* Mill.), tomato (*Lycopersicon esculentum* Mill.), turnip (*Brassica rapa* L.), Welsh onion (*Allium fistulosum* L.), black gram sprouts (*Phaseolus mungo* L.), garden pea (*Pisum sativum* L.) and snap bean (*Phaseolus vulgaris* L.). Although black gram sprouts, garden peas, and snap beans belong to another plant category, we included them in the study because they are commonly used in the Japanese diet as vegetables. Leaf vegetables (cabbage, Chinese cabbage, komatsuna, lettuce, and spinach), black gram sprouts, garden pea and snap beans were trimmed. The carrot, Japanese radish, and turnip were partly peeled. The edible burdock and onion were also peeled and their roots were removed. The calyxes of cucumber, eggplant, pumpkin, sweet pepper, and tomato were removed. The root of

Welsh onion was removed. All vegetables were frozen at approximately −40 °C and then freeze-dried at room temperature for about 1 wk (Bio Freeze Dryer-3S, Nihon Freezer Co., Bunkyo-ku, Tokyo, Japan). The freeze-dried samples were finely ground with a mill (model MX-V100, National, Osaka, Osaka, Japan) and stored in tightly sealed containers prior to analysis.

Analysis

Total nitrogen content was determined by the improved Kjeldahl method for nitrate-containing samples (AOAC 1990).

Amino acid compositions:

Amino acid analysis of each vegetable was carried out using an amino acid analyzer (Hitachi 835, Hitachi Ltd., Shibuya-ku, Tokyo, Japan) after hydrolysis with 6 N HCl at 110 °C for 24 h under vacuum. Cysteine and cystine were measured as cysteic acids (reported as Cys/2) and methionine was measured as methionine sulfone after oxidation with performic acid and hydrolysis with 6 N HCl at 150 °C for 20 h according to the method of Moore (1963). The determination of tryptophan was performed on a $Ba(OH)_2$ hydrolysate as described by Tkachuk and Irvine (1969). The amide nitrogen content was measured by titration of the ammonia liberated by hydrolysis of the sample protein with 2 N HCl (Bailey 1937) using a Conway vessel. As a blank, ammonia in a 70% ethanol extract of the samples was also measured. Free amino acids were determined by automatic amino acid analysis according to a method described previously (Sato and others 1985).

Based on amino acid analysis, we calculated the following values: The total amino acids value (total AA) was the sum of the weights of the amino acids, including asparagine and glutamine calculated from the amide nitrogen. The moles of amide nitrogen were assigned on a proportional basis to the moles of aspartic acid and glutamic acid in the samples to determine asparagine and glutamine values (Sosulski and Imafidon 1990). The free amino acid value (free AA) was the sum of the weights of each free amino acid. The amino acid residues value (AAres), was based on the molecular weight of each amino acid less the molecular weight of water, represents the true weight of protein in the sample. In this study, the AAres value by definition includes both free and bound amino acids in the total protein content. Therefore, water was subtracted from not only the bound amino acids but also from the free amino acids. Total nitrogen content in amino acids (AAN) was the corresponding weight of nitrogen in these amino acid residues.

Determination of nitrate levels:

Nitrate was measured by ion chromatography with suppressed conductivity detection. The conditions for analysis were as follows: column, Ion Pac AS12A (4.0 mm i.d. × 200 mm) (Dionex, Nippon Dionex K.K., Osaka, Japan) with Ion Pac AG12A (4.0 mm i.d. × 50 mm) (Dionex) as a guard column; eluent, 2.7 mM sodium carbonate / 0.3 mM sodium bicarbonate; flow rate, 1.5 mL/min; injection volume, 2.5μL. All measurements were carried out at room temperature. The nitrate in the samples was extracted by adding deionized distilled water and shaking for 30 min. Peak identity was confirmed by comparing the retention times of the standard and the samples; quantification was based on peak area. Nitrogen content in nitrate was calculated from the nitrate level.

Nucleic acid nitrogen ditermination

Each vegetable sample was extracted according to the method of Marshak and Vogel (1951). Four purine bases and 3 pyrimidine bases were quantitatively analyzed using high-performance liquid chromatography (Shinoda and others 1981). The nucleic acid nitrogen of each vegetable was calculated by multiplying the concentration of each base by the theoretical percentage of nitrogen.

Results and Discussion

MEASUREMENTS OF TOTAL AA, FREE AA, asparagines, and glutamine (Asn+Gln) content, AAres and AAN in vegetables are summarized in Table 1. In addition, the N:P conversion factors of the vegetables based on quantitative amino acid composition data, including an estimation of the amide nitrogen originating from Asn and Gln, were also compared. The average sum of Asn and Gln was 2.84 ± 2.208 mg (mean ± SD) per 100 g dry matter. The average value of free AA was 5.46 per 100 g dry matter. The AAres values per 100 g dry matter ranged from 4.22 g in pumpkin to 29.58 g in black gram sprouts and averaged 14.00 ± 7.165 g for the 20 vegetables. The AAN values of the 20 vegetables ranged from 0.66 g to 5.33 g per 100 g dry matter. The N:P conversion factor was then determined from the ratio of AAres to AAN in each vegetable. The resulting values ranged from 4.96 for edible burdock to 6.77 for Chinese chive. Excluding carrot, Chinese chive, Japanese radish, pumpkin, and tomato, the calculated conversion factors were below the commonly used conversion factor of 6.25. The average N:P conversion factor for the 20 vegetables was 6.00 ± 0.476 (mean ± SD). Yamaguchi and others (1978) analyzed amide nitrogen and indicated that 5.53 was a better value for vegetables. Our averaged conversion factor of 6.00, based on AAres and AAN including amide nitrogen, was higher than that value.

From the nitrate nitrogen, the nucleic acid nitrogen (NAN), and ammonia nitrogen data obtained, a table showing the distribution of nitrogen components in vegetables was constructed (Table 2). The AAN occurring in either free or bound forms ranged from 507 mg in Japanese radish to 881 mg in tomato, and averaged 731 mg per g total nitrogen, indicating that the nitrogen recovered in amino acids was 73% of the total nitrogen. The remaining 27% must be due to the presence of other nitrogenous substances, which may result in the overestimation of protein content when the common factor is used. The average recovery of AAN from total nitrogen agrees with that from a study reported previously (Tokoro and others 1987) in which a different analytical procedure was employed.

Nitrate nitrogen per g total nitrogen ranged from 1 mg in onion, black gram sprouts, and garden peas to 346 mg in Japanese radish. The value of NAN ranged from 16 mg in edible burdock to 54 mg in Welsh onion per g total nitrogen. The average level of ammonia nitrogen was 21 mg per g total nitrogen. The average sum of these nitrogen contents including AAN was 852 ± 84.5 mg (mean ± SD) per g total nitrogen. A considerable variation in the nitrate content of the vegetables was found, whereas in other nitrogenous compounds, there is almost no variability among species. The average N:P conversion factor for vegetables, calculated from their AAres and AAN, was 6.00. However, application of this factor would result in an overestimation of protein from the presence of high nonprotein nitrogen content. Although the Kjeldahl digestion method is satisfactory for determining total nitrogen, it is imprecise for determining total protein content. The presence of variable amounts of nonprotein nitrogenous components produces error if the formula (total nitrogen multiplied by 6.25)

Table 1—Calculation of N:P factor of 20 vegetables

	Total AA[a]	Free AA	Asn+Gln[b]	AAres[c]	AAN[d]	N:P factor
	(g/100g of dry basis)					(AAres / AAN)
Cabbage	17.82	6.30	4.79	15.38	2.73	5.63
Carrot	6.16	2.96	0.29	5.26	0.81	6.49
Chinese cabbage	13.76	9.65	3.77	11.82	2.11	5.60
Chinese chive	31.46	2.55	2.53	27.23	4.02	6.77
Cucumber	19.58	6.49	3.70	16.86	2.88	5.86
Edible burdock	9.57	6.47	1.92	8.32	1.68	4.96
Eggplant	13.72	3.27	1.11	11.79	1.98	5.97
Japanese radish	7.36	3.04	0.61	6.35	0.94	6.76
Komatsuna	25.32	1.93	2.06	21.48	3.53	6.08
Lettuce	14.45	7.75	6.08	12.48	2.33	5.35
Onion	11.61	6.65	2.92	10.12	1.80	5.63
Pumpkin	4.90	1.60	0.17	4.22	0.66	6.39
Spinach	27.58	3.37	2.50	23.70	3.89	6.09
Sweet pepper	11.99	3.04	1.90	10.29	1.72	5.99
Tomato	11.51	5.34	1.48	9.93	1.50	6.63
Turnip	12.10	3.38	1.57	10.40	1.68	6.20
Welsh onion	10.51	2.49	1.32	9.06	1.45	6.24
Black gram sprouts	34.25	16.16	8.28	29.58	5.33	5.55
Garden pea	23.49	13.26	6.99	20.14	3.38	5.96
Snap bean	18.09	3.53	2.88	15.50	2.66	5.82
avg	16.26	5.46	2.84	14.00	2.35	6.00
S.D.	±8.328	±3.853	±2.208	±7.165	±1.205	±0.476

[a] Total amino acids including free amino acids and Asn+ Gln.
[b] Calculated from amide-N.
[c] Total AA minus water.
[d] Nitrogen content in Total AA.

Table 2 Distribution of N components in vegetables and adjusted N:P factors

	Total N	AAN[a]	NO_3-N[b]	NAN[c]	NH_3-N[d]	Total	Adjusted N:P factor
	(% of DM[e])	(mg / g of Total N)					(AAres / Total N)
Cabbage	3.38	807	36	29	17	889	4.54
Carrot	1.07	760	53	27	16	856	4.93
Chinese cabbage	3.96	533	117	23	35	708	2.99
Chinese chive	4.75	847	72	35	17	970	5.73
Cucumber	3.62	794	32	33	20	880	4.65
Edible burdock	2.38	704	96	16	6	823	3.49
Eggplant	2.60	759	48	42	19	869	4.53
Japanese radish	1.85	507	346	20	36	909	3.43
Komatsuna	5.27	670	207	40	5	923	4.07
Lettuce	3.96	589	137	28	26	780	3.15
Onion	2.08	865	1	17	42	925	4.87
Pumpkin	0.99	668	2	39	21	730	4.27
Spinach	5.62	692	53	40	17	801	4.21
Sweet pepper	2.39	719	27	35	13	795	4.30
Tomato	1.70	881	5	27	26	939	5.84
Turnip	1.93	867	79	50	46	1041	5.38
Welsh onion	1.93	751	4	54	23	831	4.69
Black gram sprouts	6.85	779	1	18	7	804	4.33
Garden pea	4.80	704	1	29	12	745	4.20
Snap bean	3.72	717	54	43	8	823	4.17
avg	3.24	731	68	32	21	852	4.39
S.D.	±1.617	±104.4	±84.6	±10.8	±11.7	±84.5	±0.765

[a] Nitrogen content in total AA.
[b] Nitrogen content in nitrate.
[c] Nucleic acid nitrogen.
[d] Ammonia nitrogen.
[e] DM=dry matter.

is used to calculate protein content. To determine protein content more accurately using the N:P conversion factor, the total nitrogen amount should be corrected for nonprotein nitrogen content, which in vegetables accounts for 27% of the total nitrogen. Because analyzing the various kinds of nonprotein nitrogen of samples is difficult, in a previous paper (Fujihara and others 1995) we proposed an adjusted N:P conversion factor for mushrooms, taking into account nonprotein nitrogen, defined as the ratio of AAres to total nitrogen. By the same method, we calculated the adjusted N:P conversion factor for vegetables. As shown in Table 2, the adjusted N:P conversion factors of vegetables ranged from 2.99 in Chinese cabbage to 5.84 in tomato, averaging 4.39 ± 0.765. This averaged factor is similar to the factor reported by Tokoro and others (1987), based on data analysis of 12 vegetables. However, a recent study (Sosulski and Imafidom 1990) reported conversion factors much higher than those reported here: cabbage 5.30, carrot 5.80, lettuce 5.14, and tomato 6.26.

The protein contents of vegetables were obtained by five different calculations:

(1) summation of AAres content of each vegetable;

(2) multiplication of total nitrogen by 6.25, the commonly applied factor for foods;

(3) multiplication of total nitrogen by the N:P factor specific for each vegetable (see Table 1);

(4) multiplication of total nitrogen by the adjusted N:P factor, 4.39;

(5) multiplication of residual nitrogen, after subtracting nonprotein nitrogen from total nitrogen, by the N:P factor specific for each vegetable.

The results of these calculations are shown in Table 3.

The best estimation of protein content was the summation of AAres (calculation 1), which represents the true protein content in each sample. A comparison of calculated values demonstrated significant differences between AAres and the protein contents obtained by calculations (2) and (3). The protein contents obtained by these schemes were higher than the actual protein value. Except for a few vegetables, the protein contents obtained by calculation 5 (total nitrogen minus nonprotein nitrogen then multiplied by the specific N:P conversion factor) were quite similar to the corresponding AAres values. In lettuce, pumpkin, and garden peas, the calculated protein contents differed from those of their AAres. These differences might reflect the existence of other nitrogenous compounds that were not investigated in our study. Otherwise, the protein contents obtained by calculation 4 (using the adjusted N:P factor of 4.39) were in good agreement with those of their AAres.

Conclusion

These results indicate that our adjusted N:P conversion factor, that is, 4.39 is reliable and practical for estimating the protein contents of vegeta-

Table 3—Comparison of calculated protein contents in vegetables

(g / 100g of dry matter)

	AAres	Calculated protein contents			
		Total N ×6.25	Total N ×N:P factor[a]	Total N ×4.39[b]	(Protein N)[c] ×N:P factor[a]
Cabbage	15.38	21.15	19.06	14.86	17.50
Carrot	5.26	6.66	6.92	4.68	6.26
Chinese cabbage	11.82	24.75	22.17	17.39	15.05
Chinese chive	27.23	29.69	32.17	20.85	28.20
Cucumber	16.86	22.65	21.22	15.91	19.41
Edible burdock	8.32	14.90	11.82	10.47	10.42
Eggplant	11.79	16.26	15.52	11.42	13.82
Japanese radish	6.35	11.59	12.54	8.14	7.49
Komatsuna	21.48	32.96	32.09	23.15	24.00
Lettuce	12.48	24.78	21.19	17.40	17.14
Onion	10.12	13.00	11.70	9.13	11.00
Pumpkin	4.22	6.18	6.32	4.34	5.92
Spinach	23.70	35.15	34.26	24.69	30.52
Sweet pepper	10.29	14.94	14.31	10.50	13.23
Tomato	9.93	10.62	11.26	7.46	10.62
Turnip	10.40	12.09	12.00	8.49	9.91
Welsh onion	9.06	12.08	12.07	8.48	11.09
Black gram sprouts	29.58	42.74	37.92	30.02	36.96
Garden pea	20.14	30.00	28.60	21.07	27.43
Snap bean	15.50	23.22	21.62	16.31	19.32
avg	14.00	20.27	19.24	14.24	16.76
S.D.	±7.165	±10.102	±9.453	±7.095	±8.695

[a] Specific N:P factor.
[b] Averaged 'adjusted N:P factor'.
[c] Total N minus nonprotein nitrogen (NO_3-N + NAN + NH_3-N).

bles. This experiment and our previous work suggest that it is necessary to consider the often-overlooked existence of nonprotein nitrogen and amide nitrogen, which may alter the estimation of protein contents in foods. Our results also indicate that an adjusted N:P conversion factor could give a more accurate protein value. The means of calculating this factor could be applicable to many other foods, especially to foods that contain a high level of nonprotein nitrogen.

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