

# Amino Acid Composition and Nitrogen-to-Protein Conversion Factors for Animal and Plant Foods

Frank W. Sosulski\* and Gilbert I. Imafidon†

Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

Five multi-N (Gln, Asn) and basic amino acids (Lys, Arg, His) constituted one-third of the total amino acids in 23 food products. Wide variations in composition of these amino acids plus Glu, Leu, Pro, and Val had a marked influence on the nitrogen-to-protein (N:P) conversion factors. These N:P factors ranged from 6.02 to 6.15 for dairy products, from 5.61 to 5.93 for egg, meat, fish, and cereal products, and from 5.14 to 6.26 for legume, root, tuber, vegetable, fruit, and microbial foods. The average N:P factor was  $5.68 \pm 0.30$ , and a common N:P factor of 5.70 is recommended for use in all mixed or blended foods or diets where accurate protein values are required for nutritional applications.

Proteins are major structural and metabolic constituents of plant and animal materials and are important sources of dietary amino acids as well as functional components in foods. The quantitation of total protein content is a common laboratory procedure, and accuracy is essential in many food and feed applications. The Kjeldahl method for determination of total organic nitrogen (N) is a widely accepted procedure. However, the selection of the appropriate nitrogen-to-protein conversion factor (N:P factor) for calculating the total protein content has been a point of controversy, and practices are not consistent among laboratories.

A N:P Conversion Factor Committee of the Association of Official Analytical Chemists (Baker, 1982) concluded that accurate factors for conversion of N:P do not exist and reaffirmed the continued use of a set of factors compiled by the USDA (1963), as reprinted by Baker (1979) and in Method 14.068 in AOAC (1984). Except for a short list of specific factors for certain cereals, legumes, nuts, oilseeds, and milk, these references recommend the 6.25 factor for most plant and animal proteins which are assumed to contain 16% N ( $100 \div 16 = 6.25$ ) and only protein N. However, food and feed protein sources contain a complex mixture of proteins that vary in amino acid (AA) and N compositions, and their utilization is, to a large degree, in blends (Tkachuk, 1977). Therefore, the application of diverse N:P factors in feeding or processing situations has proven to be impractical, and the use of the common factor of 6.25 has often been the only reasonable alternative.

The N:P factors derived from the ratios of total amino acid residues (AAres) to amino acid N (AAN) in a protein should provide the most reliable conversion factors (Heidelbaugh et al., 1975; Tkachuk, 1977; Peace et al., 1988). In the procedure for calculation of N:P factors in cereal and oilseed proteins, Tkachuk (1969) calculated the contributions of Gln and Asn from the recovery of  $\text{NH}_3$  during AA analysis. Sosulski and Holt (1980) mea-

sured the specific amide N contents of the sample proteins by acid hydrolysis and titration of the liberated  $\text{NH}_3$ .

Recently, Imafidon and Sosulski (1990a) found that nucleic acid N from nucleoproteins represented 0.1-9.6% of total N in a wide range of plant and animal foods. Dietary nucleoproteins and nucleic acids are hydrolyzed to nucleosides and free bases in the digestive system before absorption by the intestinal mucosa to function as metabolites in the body (Lehninger, 1982). In this study, the nucleic acid N values are considered to be part of the protein complex.

To more accurately define the protein content of a food product, some investigators have recommended that nonprotein N be excluded from the Kjeldahl N value used to calculate the protein content (Tkachuk, 1977; Sosulski and Holt, 1980; Ogawa et al., 1987). The proportions of nonprotein N in primary food products vary widely, depending on sample and method of extraction, and values in the range 5-12% are not uncommon (Bhatty and Finlayson, 1973; Bhatty et al., 1973). Rigorous exclusion of polypeptides by ultrafiltration, as applied by Holt and Sosulski (1981), reduced nonprotein N to 0.0-4.4% of total N in a wide range of animal and grain products but fresh fruits, vegetables, and roots had 6.5-23.5% nonprotein N associated with low total N contents (Imafidon and Sosulski, 1990b).

Free AA and peptides in the nonprotein N can spare specific essential AA and protein in the diet (Irwin and Hegsted, 1971; Kies, 1972), and several investigators have reported that AAN constitutes over half of nonprotein N (Christianson et al., 1965; Kamppoor et al., 1975; Holt and Sosulski, 1981). In addition, nonprotein N contains numerous other nutritional or metabolic compounds that are absorbed during digestion, such as amines (decarboxylated amino acids), several vitamins, alkaloids, and ureides, and have C:N ratios in the same range as amino acids. Further, the role of nonspecific nitrogen such as urea and diammonium citrate can reduce the requirements for specific essential amino acids (Kies, 1974; Korslund, 1974). Thus, there appeared to be no sound basis for exclusion of nonprotein N from Kjeldahl N in the computation of the N:P factor.

The objective of the present investigation was to determine the specific N:P factors for a wide range of animal

\* Address correspondence to this author.

† Present address: Department of Animal Science, MacDonald College of McGill University, Ste. Anne de Bellevue, PQ, Canada H9X 1C0.

**Table I. Molecular Data on AA and Average AA Content of Animal Food Products in Milligrams per Gram of Sample N, Dry Basis**

AA	MW <sup>a</sup>	AAN <sup>b</sup>	casein	milk	cheese	egg	beef	chicken	fish
Arg	174.2	0.3215	240	234	169	470	420	406	388
His	155.2	0.2706	112	188	156	150	230	169	200
Asn	132.1	0.2118	254	292	355	324	242	213	289
Gln	146.1	0.1917	785	783	713	456	400	318	348
Lys	146.2	0.1915	520	487	400	509	556	468	538
Gly	75.1	0.1864	144	128	131	197	290	425	344
Ala	89.1	0.1571	200	219	194	327	385	287	431
Trp	204.2	0.1371	142	90	90	136	78	71	89
Ser	105.1	0.1332	385	338	306	434	300	244	294
Pro	115.1	0.1216	722	571	931	262	250	260	194
Val	117.1	0.1196	434	428	363	385	325	331	406
Thr	119.1	0.1175	294	278	194	275	270	287	289
Cys/2	121.2	0.1155	30	47	51	128	74	66	66
Ile	131.2	0.1067	345	290	281	400	340	350	300
Leu	131.2	0.1067	602	600	544	491	500	462	512
Asp	133.1	0.1052	200	214	242	266	366	421	515
Glu	147.1	0.0952	610	574	482	377	602	630	619
Met	149.2	0.0938	164	148	241	204	164	160	157
Phe	165.2	0.0847	331	341	294	294	280	275	319
Tyr	181.2	0.0773	371	297	300	266	210	225	219
total			6885	6547	6437	6351	6282	6068	6517

<sup>a</sup> Molecular weight. <sup>b</sup> Proportion.

and plant foods from their AA compositions. Data on amide N contents were used to estimate the Asn and Gln contents. Protein contents were then calculated by using total Kjeldahl N and the N:P factor with no correction for non-amino-acid N in the nonprotein N.

#### MATERIALS AND METHODS

The animal and plant food products were obtained from each of two commercial stores in Saskatoon, SK, except for casein (Sheffields Co., Norwich, NY) and the grains (Department of Crop Science, University of Saskatchewan, Saskatoon, SK). The animal products, cows' milk, cheddar cheese, blended egg white and yolk, lean muscle from beef steak, yellow perch filet, and a blend of white and brown chicken meat, were freeze-dried and defatted with hexane on a Goldfish extractor before they were ground to 0.25-mm particle size on a Krups coffee grinder. The whole grains (sorghum, wheat, corn, field pea, dry bean) and polished rice were ground directly on the Krups coffee mill. The root crops (carrot, beet), tuber (potato), leafy vegetables (lettuce, cabbage), fruits (tomato, peeled banana, apple), bakers' yeast and mushroom (*Agaricus* spp.) were freeze-dried before they were finely ground on a Udy mill to achieve the 0.25-mm particle size.

Moisture and total N contents were determined by standard procedures (AOAC, 1984) except that a 100:3 mixture of K<sub>2</sub>SO<sub>4</sub> and CuSO<sub>4</sub> was substituted for K<sub>2</sub>SO<sub>4</sub>/HgO in the micro-Kjeldahl N procedure, as applied in AOAC Method 7.033.

Fifteen AA were analyzed on a Beckman 119BL AA analyzer using the accelerated procedure of Spackman et al. (1958) after 6 N HCl hydrolysis of the samples at 110 ± 1 °C for 24 h under vacuum. Cysteine and Cys were measured as cysteine acid (reported as Cys/2), and Met was measured as methionine sulfone after performic acid oxidation and HCl hydrolysis according to the method of Moore (1963). The Trp analysis was done separately on a Ba(OH)<sub>2</sub> hydrolysate as described by Tkachuk and Irvine (1969).

The amide N contents were determined by 2 N HCl hydrolysis of the sample proteins and NH<sub>3</sub> titration (Bailey, 1967). The moles of amide N were assigned on a proportional basis to the moles of Asp and Glu present in the samples to obtain the Asn and Gln values.

The N:P conversion factors were computed from the ratios of total AA residues (AAres) to total N contents of the AA. The protein contents of the food products were calculated by these specific N:P factors as well as by the average N:P factor, 5.70.

#### RESULTS AND DISCUSSION

**Amino Acid Composition.** The compositions of 20 AA in the 23 food products, expressed as percent of sample N, are presented in Tables I–III. The AA are listed in order of decreasing proportion of N in the AA (Table I).

The average concentrations of the AA in dairy, egg, and meat products (Table I) were similar to the mean values compiled by the FAO (1970) and specific data of Holt and Sosulski (1974), Heidelbaugh et al. (1975), and Mai et al. (1980), except for higher average recoveries in this study. Most investigators did not estimate the Asn and Gln values, and methods for determination of Trp, Cys/2, and Met were not the same. Gln, Pro, Leu, Glu, Lys, and Val constituted over half of the total AA in casein, milk, and cheese, while egg exhibited a much more even distribution of AA. Glu, Lys, and Leu were major AA in the meat and fish products.

The AA profiles for wheat, rice, corn, and sorghum (Table II) were also in agreement with the FAO (1970) averages and the patterns obtained by Tkachuk and Irvine (1969) for cereals, by Bradbury et al. (1980) for rice, and by Sikka and Johari (1979) for sorghum. There are numerous literature values for AA composition of field peas and dry beans. The present values are close to those of Sosulski and Holt (1980) and Tkachuk (1977), in which similar analytical procedures were employed. Gln, Pro, and Leu were the principal amino acids in wheat, corn, and sorghum, while Gln, Arg, and Leu predominated in rice. Basic and multi-N AA were major contributors of AAN in the legumes, field pea and dry bean, with Arg, Gln, Asn, and Lys being the most important AA. However, the contents of total sulfur-containing AA, Cys/2 and Met, were much less than occurred in the animal and cereal products.

As in the grains, Gln and/or Glu were the predominant AA in the root crops, carrot and beet (Table II). However, the carrot displayed a much better balance of essential AA such as Lys, Met, Arg, Ile, and Phe than was found in beet. As shown later, the carrot contained much less total N than the beet or potato, while the latter were characterized by generally lower recoveries of

Table II. Average AA Content of Cereal, Legume, Root, and Tuber Products in Milligrams per Gram of Sample N, Dry Basis

AA	wheat	rice	corn	sorghum	field pea	dry bean	carrot	beet	potato
Arg	252	488	262	241	586	388	325	221	239
His	157	159	213	138	145	188	144	119	110
Asn	246	326	392	375	390	531	297	320	966
Gln	1398	652	891	819	561	780	517	1609	852
Lys	188	250	167	132	444	419	456	213	250
Gly	266	269	269	192	278	225	272	143	171
Ala	244	344	471	542	267	231	322	257	218
Trp	53	57	59	75	72	81	56	47	59
Ser	315	253	362	271	271	344	253	243	226
Pro	714	257	606	501	222	256	225	125	179
Val	281	434	303	364	300	294	382	208	329
Thr	191	230	256	196	247	231	241	161	275
Cys/2	164	129	129	131	81	75	99	74	56
Ile	288	231	200	226	253	281	350	184	211
Leu	450	485	783	900	443	488	253	256	281
Asp	92	221	105	185	333	196	378	57	52
Glu	521	444	241	400	480	284	655	284	49
Met	92	133	126	90	51	81	98	51	63
Phe	316	366	308	302	284	356	288	163	226
Tyr	229	204	239	225	170	181	187	160	133
total	6457	5932	6382	6305	5878	5910	5798	4895	4945

Table III. Average AA Content of Fresh Vegetable, Fruit, and Microbial Food Products in Milligrams per Gram of Sample N, Dry Basis

AA	lettuce	cabbage	tomato	banana	apple	yeast	mushroom	mean <sup>a</sup>
Arg	257	500	263	521	244	224	172	326.5
His	108	138	68	450	194	116	103	163.3
Asn	683	366	373	289	622	417	425	390.7
Gln	1207	931	757	222	387	541	729	724.2
Lys	281	215	244	270	476	455	275	357.1
Gly	191	171	150	234	259	268	184	226.1
Ala	291	242	144	312	328	268	291	296.3
Trp	67	96	64	45	59	70	78	75.4
Ser	278	188	163	246	265	284	200	281.0
Pro	170	208	142	240	277	211	188	335.3
Val	268	219	181	200	450	295	206	321.1
Thr	225	171	163	200	212	291	369	241.1
Cys/2	72	79	93	161	101	120	58	90.6
Ile	190	160	131	182	228	256	156	253.6
Leu	301	230	188	294	447	397	259	442.0
Asp	32	225	849	503	405	346	44	271.6
Glu	54	574	1720	390	252	447	75	468.0
Met	53	39	48	111	58	94	74	108.7
Phe	190	168	200	240	379	246	175	275.7
Tyr	145	114	119	160	265	170	431	218.3
total	5063	5034	6060	5270	5908	5516	4492	5866.6

<sup>a</sup> Mean for 23 food products in Tables I-III.

total AA. There is substantial information in the literature on the AA composition of the potato, and the present results are in line with the averages reported by FAO (1970). Like lettuce leaves and apple fruit, the concentration of Asn in the potato exceeded that of Gln.

Analysis of the free and protein AA in the two leafy vegetables indicated that Gln was the major AA but the amide N content of lettuce was greater than in cabbage (Table III). Thus, the Asn level in lettuce was comparatively higher than in cabbage, but cabbage contained more Glu and Arg.

Among the fruits, tomato was particularly rich in Glu and Asp, as well as Gln and Asn, but His and Met contents were very low (Table III). Banana had high Arg and His levels but low Trp content, while apple showed more Lys than the other fruits and vegetables. Bakers' yeast also contained a high level of Lys. The total recovery of AA from mushroom was the lowest of all samples, less than 4500 mg/g of sample N, and so the concentra-

tion of most AA appeared relatively low. Threonine and Tyr levels in mushroom, however, exceeded those of all other foods in the experiment. The low recovery of AA, and more even distribution of AA, in cultivated mushrooms was also reported by Ogawa et al. (1987), who detected a significant quantity of glucosamine which was derived from chitin-like polysaccharides.

On the average, the five multi-N (Gln, Asn) and basic AA (Lys, Arg, His) constituted one-third of the total AA in the 23 food products (Table III). In addition, the ranges in composition of each of these AA among the food products were also relatively large, so their proportions in the proteins and nonprotein N would have a marked influence on the N:P factor, as calculated in the next section. The ratios of Gln and Glu and Asn to Asp would have a particularly large influence on the overall C:N ratio and the N:P factor. However, Holt and Sosulski (1979) found that N:P factors in three field pea experiments were 5.49, 5.52, and 5.53 with coefficients of variation of 1.5-1.6%

Table IV. Calculation of N:P Factors and Protein Contents of 23 Primary Food Products

food products by classes	total AA content, mg/g of product N	total AA res, mg/g of product N	total AAN, mg/g of product N	N:P factor ratio	protein content in %		total AA content, g/100 g of product N
					specific factor	N × 5.70	
dairy products and egg							
casein	6885	5967	970	6.15	84.56	78.38	82.05
milk	6547	5676	943	6.02	24.92	23.60	23.50
cheese	6437	5569	908	6.13	39.48	36.71	35.86
egg	6351	5493	958	5.73	44.41	44.18	42.57
meat and fish products							
beef	6282	5421	947	5.72	70.01	69.77	66.35
chicken	6068	5228	898	5.82	63.79	62.47	57.30
fish	6517	5619	965	5.82	74.79	73.25	72.20
cereals and legumes							
wheat	6457	5573	970	5.75	11.04	10.94	10.70
rice	5932	5128	914	5.61	5.27	5.36	4.82
corn	6382	5485	958	5.72	8.98	8.95	8.61
sorghum	6303	5427	915	5.93	11.74	11.29	10.75
field pea	5878	5082	941	5.40	19.06	20.12	17.94
dry bean	5910	5111	939	5.44	20.67	21.66	19.42
roots and tuber							
carrot	5798	5007	863	5.80	3.65	3.59	3.15
beet	4894	4238	805	5.27	8.96	9.69	7.20
potato	4945	4273	825	5.18	10.77	11.86	8.89
leafy vegetables							
lettuce	5063	4371	850	5.14	13.16	14.59	11.19
cabbage	5034	4363	823	5.30	12.72	13.68	10.47
fruits							
tomato	6060	5264	841	6.26	12.39	11.29	10.42
banana	5270	4550	856	5.32	3.88	4.16	3.32
apple	5905	5100	892	5.72	1.20	1.20	1.07
microbial							
yeast	5516	4755	823	5.78	33.12	32.66	27.25
mushroom	4492	3879	691	5.61	32.15	32.66	22.23
av	5866.3	5068.7	891.1	5.68	26.55	26.18	24.23

Table V. Distribution of N Components in 23 Food Products, Dry Basis

food products by classes	total N, % of DM	amide N, mg/g of N	total AAN, mg/g of N	NAN, <sup>a</sup> mg/g of N	NAAN, <sup>b</sup> mg/g of N	total N, mg/g of N
dairy products and egg						
casein	13.75 ± 0.09	102	970	1	0	971
milk	4.14 ± 0.03	106	943	4	27	974
cheese	6.44 ± 0.04	106	908	2	26	936
egg	7.75 ± 0.05	78	958	1	4	963
meat and fish products						
beef	12.24 ± 0.08	64	947	4	15	966
chicken	10.96 ± 0.07	53	898	5	10	913
fish	12.85 ± 0.08	64	965	3	14	982
cereals and legumes						
wheat	1.92 ± 0.01	160	970	21	12	1003
rice	0.94 ± 0.01	97	914	25	11	950
corn	1.57 ± 0.01	127	958	21	17	996
sorghum	1.98 ± 0.01	118	915	21	8	944
field pea	3.53 ± 0.02	95	941	15	35	991
dry bean	3.80 ± 0.02	131	939	20	27	986
roots and tuber						
carrot	0.63 ± 0.00	81	863	67	117	1047
beet	1.70 ± 0.01	188	805	32	192	1029
potato	2.08 ± 0.01	184	825	13	194	1032
leafy vegetables						
lettuce	2.56 ± 0.02	188	850	28	165	1043
cabbage	2.40 ± 0.02	128	823	27	186	1036
fruits						
tomato	1.98 ± 0.01	112	841	14	174	1029
banana	0.73 ± 0.00	52	856	70	125	1051
apple	0.21 ± 0.00	103	892	96	57	1045
microbial						
yeast	5.73 ± 0.04	96	823	119	70	1012
mushroom	5.73 ± 0.03	115	691	150	148	989
av	4.59	110.8	891.1	33.0	71.0	995.1

<sup>a</sup> Nucleic acid N (NAN) from nucleoproteins. <sup>b</sup> Non-amino-acid N (NAAN) from nonprotein N (Imafidon and Sosulski, 1990a,b).

among the 17, 16, and 11 samples and cultivars, respectively. The low degree of variation in N:P factor occurred because Arg content was positively correlated with vari-

ations in protein content but Gln and Asn levels were negatively associated with protein and Arg levels, and so the contents of basic and multi-N AA were relatively con-

stant in all field pea proteins.

The contents of total AA varied between 6068 and 6885 mg/g of product N among the dairy, egg, and meat products (Table IV). For the grain crops, the values were somewhat lower, the range being 5878–6457 mg/g. Total AA contents of most fresh vegetables and fruits were quite low at 4894–6060 mg/g. Mushroom also exhibited a low total AA content.

**Calculation of N:P Factors.** The AA res values (Table IV) were based on the molecular weight (MW) of each amino acid (Table I) less the MW of H<sub>2</sub>O. The concentrations of total AAres varied from 3879 (mushroom) to 5967 (casein) mg with a mean of 5069 mg/g of product N (Table IV). The total AAN per gram of product N was calculated from the AAN values (Table I) and the individual AA concentrations in each product. The N:P factor was then determined from the ratio of total AAN to AAres in each product. The N:P factors for dairy products ranged from 6.02 to 6.15, while those of egg, meat, and cereals varied from 5.61 to 5.93. The legumes, fresh vegetables and fruits, and microbial products exhibited a wider range in N:P factors, from 5.14 (lettuce) to 6.26 (tomato).

These specific N:P factors were multiplied by the total (Kjeldahl) N values (Table V), in percent of dry matter (DM), to obtain the protein percentages listed in Table IV. The protein contents ranged from 1.20% (apple) to 84.56% (casein) for all food products.

The average of the N:P factors for the 23 foods was  $5.68 \pm 0.30$ , or 5.69 if calculated from the total AAres and total AAN (5068.7:891.1) (Table IV). In mixed diets or blended food products, the weighed average N:P factor would likely be close to these mean values. Therefore, the common N:P factor of 5.70 was also used to compute the protein contents that, in this case, ranged from 1.20% to 78.38%, which were close to those obtained by using specific factors. The principal discrepancy was with casein, which had the highest N:P factor and the highest N content. The correlation coefficient between protein contents of the 23 food products calculated by the specific and common N:P factors was  $r = 0.999^{**}$  ( $df = 21$ ).

The total AA content of each food product, in grams of AA per 100 g of product, was calculated to compare with the protein percentages in Table IV. The range in values was from 1.07 to 82.05 g with a mean of 24.23 g. Essentially all totals were slightly lower than protein percentages based on either N:P factor. An exception was casein at 82.05 g of total AA per 100 g of product, which exceeded the protein value obtained by use of the 5.70 factor. The generally lower AA contents relative to calculated protein percentage was due, in part, to incomplete recovery of AA or AA losses during hydrolysis and the presence of nucleic acid N and non-amino-acid N.

By use of nucleic acid N and non-amino-acid N data from Imafidon and Sosulski (1990a,b), it was possible to structure a general table of N components in the 23 food products (Table V). When the amide N values (average 110.8 mg/g of N) were combined with the AA data, the total AAN averaged 891.1 mg/g of N with only mushroom having a value below 800 mg. The previously published values for nucleic acid N (33.0 mg/g of N) and non-amino-acid N (71.0 mg/g of N) brought the average total recovery to 995.1 mg/g of N, with many totals being slightly above 100% recovery.

#### ACKNOWLEDGMENT

We are indebted to the Natural Sciences and Engineering Research Council for financial support.

#### LITERATURE CITED

- AOAC. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th ed.; Association of Official Analytical Chemists: Arlington, VA, 1984.
- Bailey, J. L. *Techniques in Protein Chemistry*; Elsevier Publishing: Amsterdam, 1967.
- Baker, D. Report on cereal foods, General Referee Reports. *J. Assoc. Off. Anal. Chem.* **1979**, *62*, 369–370.
- Baker, D. Report on cereal foods, General Referee Reports. *J. Assoc. Off. Anal. Chem.* **1982**, *65*, 333–334.
- Bhatty, R. S.; Finlayson, A. J. Extraction of nonprotein nitrogen from oilseed meals with different solvents. *Cereal Chem.* **1973**, *50*, 329–336.
- Bhatty, R. S.; Sosulski, F. W.; Wu, K. K. Protein and nonprotein nitrogen contents of some oilseeds and peas. *Can. J. Plant Sci.* **1973**, *53*, 651–657.
- Bradbury, J. H.; Collins, J. G.; Pylotitis, N. A. Amino acid analyses of the proteins of the major histological components of a high-protein rice. *Cereal Chem.* **1980**, *57*, 343–346.
- Christianson, D. D.; Wall, J. S.; Cavins, J. F. Nutrient distribution in grain. Location of nonprotein nitrogenous substances in corn grain. *J. Agric. Food Chem.* **1965**, *13*, 272–276.
- Food and Agriculture Organization of the United Nations (FAO). Amino acid content of foods and biological data on proteins, Nutrition Studies Report No. 24, Rome, 1970.
- 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. Proximate and amino acid composition of ground meat products. *Can. Inst. Food Sci. Technol. J.* **1974**, *7*, 144–147.
- 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.
- Imafidon, G. I.; Sosulski, F. Nucleic acid nitrogen in animal and plant foods. *J. Agric. Food Chem.* **1990a**, *38*, 118–120.
- Imafidon, G. I.; Sosulski, F. W. Nonprotein nitrogen contents of animal and plant foods. *J. Agric. Food Chem.* **1990b**, *38*, 114–118.
- Irwin, M. I.; Hegsted, D. M. A conspectus of research on protein requirements of man. *J. Nutr.* **1971**, *101*, 385–429.
- Kapoor, A. C.; Desborough, S. L.; Li, P. H. Extraction of nonprotein nitrogen from potato tuber and its amino acid composition. *Potato Res.* **1975**, *18*, 582–587.
- Kies, C. Nonspecific nitrogen in the nutrition of human beings. *Fed. Proc.* **1972**, *31*, 1172–1177.
- Kies, C. Comparative value of various sources of nonspecific nitrogen for the human. *J. Agric. Food Chem.* **1974**, *22*, 190–193.
- Korslund, M. K. Nonessential nitrogen utilization by children and adolescents. *J. Agric. Food Chem.* **1974**, *22*, 187–189.
- Lehninger, A. L. *Principles of Biochemistry*; Worth Publishers: New York, 1982.
- Mai, J.; Shetty, J. K.; Kan, T. M.; Kinsella, J. E. Protein and amino acid composition of selected fresh-water fish. *J. Agric. Food Chem.* **1980**, *28*, 884–885.
- Moore, S. On the determination of cystine as cysteic acid. *J. Biol. Chem.* **1963**, *238*, 235–237.
- Ogawa, T.; Oka, Y.; Sasoaka, K. Amino acid profile of common cultivated mushrooms including the identification of N-(N-r-L-glutamyl-3-sulfo-L-alanyl) glycine in *Flammulina velutipes*. *J. Food Sci.* **1987**, *52*, 135–137, 154.
- Peace, R. W.; Keith, M. O.; Sarwar, G.; Botting, H. G. Effects of storage on protein nutritional quality of grain legumes. *J. Food Sci.* **1988**, *53*, 439–459.
- Sikka, K. C.; Johari, R. P. Comparative nutritive value and amino acid content of different varieties of sorghum and effect of lysine fortification. *J. Agric. Food Chem.* **1979**, *27*, 962–965.

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. Spackman, D. H.; Stein, W. H.; Moore, S. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* 1958, 30, 1190-1206.

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., Billingsley, L. W., Cochairmen; International Development Research Centre: Ottawa, 1977; pp 78-82.

Tkachuk, R.; Irvine, G. N. Amino acid composition of cereals and oilseed meals. *Cereal Chem.* 1969, 46, 206-218.

USDA. *Composition of Foods*; Handbook 8; U.S. Department of Agriculture: Washington, DC, 1963.

Received for review August 21, 1989. Revised manuscript received February 6, 1990. Accepted February 13, 1990.

**Registry No.** Gln, 56-85-9; Asp, 70-47-3; Lys, 56-87-1; Arg, 74-79-3; His, 71-00-1; Glu, 56-86-0; Leu, 61-90-5; Pro, 147-85-3; Val, 72-18-4; N, 7727-37-9.

## Determination of Dextrose Equivalent in Starch Hydrolysates Using Cerium(IV)

Lee S. Griffith and Peter Sporns\*

Department of Food Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

The time for reduction of a fixed amount of Ce(IV) by an excess of carbohydrate solution (4.0% w/v) was dependent on the concentration of reducing sugar in the solution. The colorimetric conversion of Ce(IV) to Ce(III) could be observed visually or, for more accurate determinations, with a spectrophotometer set to monitor the absorbance change at 445 nm. The inverse of the Ce(IV) reduction time gave an excellent linear correlation ( $r = 0.995$ ) with the dextrose equivalent (DE) of starch hydrolysate solutions as measured by using the Lane Eynon method. The Ce(IV) test was simple, rapid, and inexpensive and offered an alternative method for the determination of DE in process control of starch hydrolysis.

Although time-consuming, the Lane Eynon or similar copper complex oxidation-reduction reactions have been the traditional and official methods for measuring the dextrose equivalent (DE) of starch hydrolysates (De Whalley, 1964). DE is an important property of starch hydrolysates, being an indirect measure of the degree of hydrolysis of the starch. Higher hydrolyzed products are sweeter and more soluble in water and have different effects upon the rheology and texture than products of lower starch hydrolysis. A product with a high DE has a higher degree of hydrolysis than a product with a lower DE. Numerous other methods, notably, freezing point depression (cryoscopy) and high-performance liquid chromatography (HPLC), have been introduced as possible replacements for the traditional quality control methodology (Delheye and Moreels, 1988). Although rapid, cryoscopy requires relatively expensive equipment and is affected by a variety of production parameters such as the raw material, production method, purification technique, and salt content. HPLC gives quantitative information on oligosaccharides present but also requires expensive equipment, greater expertise, time, and conversion of each oligosaccharide with a factor to establish the overall DE of the hydrolysate.

While working on a method for measuring lactose hydrolysis in milk, Griffith et al. (1989) noted that hydrolysis

to monosaccharides could be measured by determining the increasingly rapid reduction of cerium(IV) to cerium(III) using an excess of carbohydrate. This paper investigates the use of this cerium reaction to measure the DE of starch hydrolysates.

### EXPERIMENTAL PROCEDURES

**Materials.** Carbohydrate standards (glucose and glucose oligosaccharides) were purchased from Sigma Chemical Co., St. Louis, MO. Starch hydrolysate materials were provided courtesy of American Maize Products Co., Hammond, IN; A. E. Staley Manufacturing Co., Decatur, IL; and Champlain Industries Ltd., Mississauga, ON. Water was prepared by using a Millipore Milli-Q system, and all other chemicals used were of reagent grade or better.

**Ce(IV) Oxidations of Carbohydrates.** Oxidations were carried out in a manner similar to the method reported by Griffith et al. (1989) except that the time in seconds was recorded for the absorbance at 445 nm to return to 0.5 OD rather than 0.4 OD. The rationale for the choice of 445 nm is explained in the earlier work of Griffith et al. (1989).

The method involved addition of 0.4 M ammonium hexanitratoceria(IV)  $[(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6]$  in 0.5 M nitric acid to carbohydrate solution in a 1:3 ratio. The Ce(IV) solution was aged at least 6 h and shaken before use. The carbohydrate solution was prepared at a concentration of  $2.00 \pm 0.01$  g in 50 mL of water, or equivalent (for carbohydrate standards, less carbohydrate in proportionately less water was used). The mixed solution was placed in a Pye-Unicam SP 1800 spectrophotometer (sugar solution as reference sample) and the time in seconds

\* Author to whom correspondence should be addressed.