Comparison of the Amino Acid Composition and Protein Contents of Two Northern Adapted Asparagus Cultivars†

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Amino acid composition, total protein content, and the separated ethanol-soluble and ethanol-insoluble protein fractions of two new asparagus cultivars were compared as potentially useful indices for evaluating their protein quality. Total protein on a dry weight basis for Jersey Centennial and Lucullus Mid-season cultivars averaged 23.1 and 24.4%, respectively. Mean values for total EAA_7 (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) and EAA_{10} (these seven, plus tryptophan, histidine, and arginine) ranged, respectively, from 28.6 to 37.6% and from 43.2 to 47.3% in the asparagus spears evaluated. The calculated protein efficiency ratios (PER) ranged from 2.1 to 2.8. The ethanol-insoluble protein fraction contained protein-bound 4-hydroxyproline. Lucullus Mid-season had greater amounts of 4-hydroxyproline than Jersey Centennial. The 4-hydroxyproline-rich glycoprotein content of the primary cell walls of asparagus spears has been determined from the amounts of 4-hydroxyproline found in their acid hydrolysates. The 4-hydroxyproline-rich glycoproteins found in the extracellular matrices of asparagus spears ranged from 0.63 to 0.96% of the total protein for Jersey Centennial and Lucullus Mid-season, respectively.

INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is a perennial crop relatively well adapted to temperate climates. However, exposure of growing spears to temperatures near or below 0 °C can result in reductions in size and spear number, which in turn reduce total yield. Unfortunately, in Canada the risk of exposure to low temperatures (≤0 °C) is high during the early spring and harvest periods. To reduce such economic losses to the asparagus industry, various new all-male hybrid cultivars with greater cold tolerance and higher productivity have been developed in several countries (Robbins and Jones, 1926, 1928; Tiessen, 1985; Burrows et al., 1989; Ellison and Kinelski, 1985; Ellison et al., 1960, 1989, 1990; Maurer et al., 1989; Wolyn et al., 1989a,b).

Extensive field trials have been carried out in Canada to identify the most productive and cold tolerant of these new cultivars (Tiessen, 1985; Wolyn et al., 1989b,c; Maurer and Onsorge, 1989; Maurer et al., 1989; Schumacher and Baker, 1989; Minero-Amador et al., 1989a–c). These studies have been primarily concerned with horticultural and postharvest aspects of this crop and report on total and marketable yields and quality. Little quantitative information is available, however, on complete amino acid composition, on protein content of asparagus, or on the effects of low temperatures on the amino acid composition.

The ability of plants to adapt to low temperatures has been attributed to changes in specific biochemical processes (Caldwell, 1990), including the synthesis of temperature-shock proteins (Guy et al., 1985), alterations in structure and function of enzymes involved in key metabolic reactions (Berry and Bjorkman, 1980; Colby and Pierce, 1988), and the development and repair of the photosynthetic apparatus (Goldberg et al., 1989; Nie and Baker, 1991). Changes in cell plasma membrane structure (Steponkus, 1984; Steponkus and Lynch, 1989; Raison and Orr, 1990) or in the insoluble cell wall proteins may also be involved in plant cell acclimation to temperature extremes (Lamport, 1977, 1980; Cassab and Varner, 1987, 1988). The work of Lamport (1977, 1980) demonstrated that the extracellular matrices of the primary cell walls of angiosperms comprise 4-hydroxyproline-rich glycoproteins, i.e., extensins, arabinogalactan proteins, salt-extractable glycoproteins, lectins, and agglutinins, in which a number of proline residues are posttranslationally modified to 4-*trans*-hydroxyproline (Lamport, 1980; Fincher et al., 1983; McNeil et al., 1984; Cassab et al., 1985; Cooper et al., 1987; Corbin et al., 1987; Averyart-Fullard et al., 1988; Showalter et al., 1990; Marcus et al., 1991; Kielszewski et al., 1992). Accurate measurements of amino acid and protein variations, including 4-hydroxyproline, in asparagus cultivar tissues would be useful as a data base on asparagus composition and hence for evaluating its protein quality.

The present study was designed to quantitatively measure the levels and variation of total protein as well as the individual amino acids in two northern adapted asparagus cultivars. The cultivars were Jersey Centennial (Rutgers University, New Jersey) and Lucullus Mid-season (Sudwest Deutsche Saatzutcht, Germany).

The aims of the present study were (1) to assess the amino acid and protein contents of two northern adapted asparagus cultivars, (2) to determine whether amino acid levels could be used as accurate measures of nutritional quality of the protein, and (3) to ascertain the total amount of 4-hydroxyproline-rich glycoproteins found in the extracellular matrices of the primary cell wall of asparagus spears.

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† Contribution No. 1420 from Plant Research Centre.

0021-8561/92/1440-2395$03.00/0

MATERIALS AND METHODS

Materials. Types DC-6A and DC-5A cation-exchange spherical resins, sized to 11.0 ± 1.0 and 6.0 ± 0.5 μm, respectively, were purchased from Dionex Chemical Co., Sunnyvale, CA. The amino acid standards were obtained as follows: L-tryptophan, D-glucosamine monohydrochloride, D-galactosamine monohydrochloride, and 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; DL-ornithine (5-aminonorvaline), cysteic acid, and methionine *S,S*-dioxide from Schwartz/Mann, Orangeburg, NY; Type H amino acid calibration mixture, norleucine, and L-2-amino-3-guanidinopropionic acid, Piercesolve (ethyl glycol monomethyl ether), ninhydrin, and stannous chloride from Pierce Chemical Co., Rockford, IL; and 3-nitro-L-tyrosine from Aldrich Chemical Co., Milwaukee, WI. High-purity hydrochloric acid (35.0%) used in the preparation of triple-glass-distilled constant-boiling (20.5%; 5.7 M) reagent (AOAC, 1984), hydrobromic acid (49.0–51.0%), hydrogen peroxide solution (30.0%), and 88% formic acid used for oxidations (Moore, 1963) were of Aristar grade from BDH Inc., Associate of E. Merck Darmstadt, Germany. The Type I standard amino acid calibration mixture was purchased from Beckman Instruments, Inc., Palo Alto, CA. All reagents and buffers were made with high-purity laboratory water (Zarkadas et al., 1987) using activated carbon beds, mixed ion exchangers, glass distillation, and deionization steps. All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Collection of Plant Materials and Sample Preparation.* The asparagus cultivar trial was established in 1985 at Macdonald College of McGill University, and the asparagus was harvested for the first season in 1988. Two cultivars, Jersey Centennial (Rutgers University, New Jersey) and Lucullus Mid-season (Sudwest Deutsche Saatzutcht, Germany), were used. The trial was laid out as randomized complete block design with four replications. Each plot consisted of a single 3.4-m row with 12 plants spaced 0.31-m within the row. Plots were separated by 1.22-m space (Minero-Amador et al., 1989a).

Spears were harvested when the head reached a minimum length of 21 cm and a base diameter greater than 7.8 mm. After harvest, all spears were trimmed to a length of 21 cm. Spears were graded as follows: No. 1, marketable large spears, minimum basal diameter 11.1 mm; No. 2, marketable medium spears, minimum basal diameter 7.9–11.1 mm; and culls, basal diameter less than 6.4 mm or damaged. Spear counts and weights in each grade were recorded. Plant survival was also recorded at harvest. Harvest started April 27 and finished June 7, 1988.

The four replicate samples from each cultivar were harvested on May 25, 1988. Samples (200 g) were randomly selected, cleaned, cut into small cubes, ground, frozen (−173 °C), and lyophilized. All tissues were ground in a standard electrically driven Thomas-Wiley Intermediate Model mill equipped with a 64-mm stainless steel hopper and stationary blades (A. H. Thomas Co., Philadelphia, PA), and stored in small plastic bottles at −20 °C for subsequent analysis. Fresh asparagus specimens of 20.0–23 cm in length were used for the preparation of extracted fractions.

Extraction Procedure and Preparation of Ethanol-Soluble (F1) and Ethanol-Insoluble (F2) Protein Fractions from Asparagus Spears. To effectively remove all traces of soluble amino acids and peptides, including proline and 4-hydroxyproline, 0.5-g samples of the pulverized asparagus spears selected were homogenized with 50 mL of 70% (v/v) ethyl alcohol, as described by Asconas (1951) and Scopes (1978, 1982), in a VirTis homogenizer (Model 23; VirTis, Gardiner, NY) for 10 s (full speed) at 2 °C. The homogenates were centrifuged at 5000*g* (SS-34 Sorvall rotor) for 15 min at 2 °C. The supernatants, designated ethanol-soluble fraction F1, were decanted, combined, dried under vacuum on a rotary evaporator (Buchi, Rotavapor, Switzerland) at 45 °C, resuspended in double-distilled water, lyophilized, and stored at −70 °C. The remaining pellet, designated 70% ethanol-insoluble fraction F2, was suspended in the same extraction solvent, and the extraction procedure was repeated twice. The combined pellets were suspended in distilled water and lyophilized. The dried pellets were then finally ground to pass through a 40-mm screen and were stored at −70 °C until needed.

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman Model 120C) or a fully automated amino acid analyzer (equivalent to Beckman Spinco Model 121MB). The automated instrument was equipped with a Varian Vista 402 chromatographic data reduction system (Varian Instruments Group, Walnut Creek, CA) to increase the sensitivity of the analysis and to enable quantitation of amino acids at the picomole level as described previously (Zarkadas et al., 1987).

Complete amino acid analyses were carried out on each of the four replicate asparagus samples (50.0 mg) per cultivar according to the standard procedures described previously (Zarkadas et al., 1986, 1987). Each of the two major fractions, F1 and F2, prepared from three different plots, were also analyzed according to the same procedure. Duplicate plant tissue samples (0.05 g) were hydrolyzed for 24, 48, 72, and 96 h, respectively, in Pyrex test tubes (18 × 150 mm) under vacuum (below 10 mmHg) with 10 mL of triple-glass-distilled constant-boiling HCl (5.7 M) at 110 °C, with the usual precautions described by Zarkadas et al. (1988a–c; Berg, 1982; Ozols, 1990). The small amounts of insoluble materials which formed during acid hydrolysis were removed by filtration (0.22-μm Millipore microfilters; Millipore Corp., Bedford, MA) and were washed with the same acid (6.0 M HCl). Foaming of hydrolysates was suppressed during evacuation by the addition of 5–10 μL of octanoic acid. The clear filtrate and washings were combined, evaporated to dryness in a Rotary Evapomix (Buchler Instruments, Fort Lee, NJ) at 45 °C, and brought to volume with 0.2 M sodium citrate buffer, pH 2.2. Norleucine and 3-nitrotyrosine, selected as the internal standards, were included in this step or prior to hydrolysis (Zarkadas et al., 1987, 1988c).

All samples were analyzed by the standard procedure (Zarkadas et al., 1990, 1992). The data reported for serine and threonine represent the average of values from 48 determinations extrapolated to zero time of hydrolysis by linear regression analysis. Addition of phenol (20–25 μL) to the hydrolysates prevented chlorination of tyrosine. The values for valine, isoleucine, leucine, and phenylalanine were averages of data from 48, 72, and 96 h of hydrolysis (36 determinations). All others were reported as the average values from 24, 48, 72, and 96 h of hydrolysis (48 determinations).

4-Hydroxyproline was determined separately, in duplicate, from six concentrated 24-h hydrolysates (equivalent to 0.1 mg of protein/analysis) using a single column (21 × 0.6 cm) packed with Dionex-type DC-6A resin (Zarkadas et al., 1986). Recoveries of 4-hydroxyproline were calculated relative to alanine, valine, isoleucine, and leucine (Zarkadas, 1992).

Methionine and cyst(e)ine were determined in duplicate from three separate samples (0.05 g) as their oxidation products by the performic acid procedure of Moore (1963). The recovery of cystine plus cysteine as cysteic acid and of methionine as the methionine *S,S*-dioxide was calculated relative to the yields obtained by the performic acid treatment of standard solutions of these amino acids and relative to the levels of alanine, valine, isoleucine, and leucine present in the samples (12 determinations).

Tryptophan in the asparagus spear samples (0.05 g) was determined separately after alkaline hydrolysis (Hugli and Moore, 1972) by an improved chromatographic procedure using 3-nitrotyrosine [Tyr(NO_2)] as an internal standard (Zarkadas et al., 1986).

Calculation of the Protein Mass in the Asparagus Tissues. The protein mass of each acid hydrolysate was determined according to the procedure described by Horstmann (1979). This method, based upon knowledge of the amino acid composition, yields accurate estimates of the amount of protein present. According to this method, a mean residue weight (WE, in micrograms per nanomole) could be calculated for the amino acids constituting the proteins in asparagus as

$$\mathrm{WE} = \sum_{i=1}^{18} (a_i b_i) \qquad (1)$$

where a_i is the mole fraction of an amino acid i found in the analyzed aliquot and b_i is the molecular weight of amino acid residue i as described by Horstmann (1979). A conversion factor (CF), which is the apparent average residue molecular weight

increased in proportion to the missing tryptophan and cyst(e)ine values and which is characteristic for each protein or protein mixture, can be calculated from the following expression:

$$CF = WE/(1 - (a_{Trp} + a_{Cys})) \quad (2)$$

A conversion factor CF′ (micrograms per nanomole) was also calculated according to eq (2) but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and Pro(4-OH). These factors (CF and CF′) are constants characteristic of each protein or protein mixture.

The amount of protein (P, in milligrams) in each hydrolysate can then be calculated as

$$P = CF\sum_{i=1}^{16} x_i \quad (3)$$

where x_i represents the nanomoles of each amino acid i found in the analyzed aliquot.

Determination of 4-Hydroxyproline-Rich Glycoproteins. In this study, an attempt was made to relate the amounts of protein-bound 4-hydroxyproline, which occurs exclusively in the 4-hydroxyproline-rich glycoproteins of the primary cell walls of the angiosperms, i.e., extensin, arabinogalactan protein, and salt-extractable glycoproteins (Lamport, 1980; Cooper et al., 1987; Cassab and Varner, 1988), to the contents of these cell wall proteins in asparagus spears. The most abundant 86-kDa carrot extensin monomer has been the best characterized (Varner and Lin, 1989), and its amino acid composition and primary sequence have been reported (Stuart and Varner, 1980; Van Holst and Varner, 1984; Chen and Varner, 1985a,b; Smith et al., 1986).

Previous studies (Zarkadas et al., 1988a) have shown that a method of calculating the amount of a specific protein (j) in plant tissues from the quantitative determination of a given unique amino acid i known to occur exclusively in that specific protein (j) is

$$P_j = c_i(1000/n'_i)(WE_{P_j}/M_{r(i)}) \quad (4)$$

where P_j is the concentration of a specific primary cell wall glycoprotein j, i.e., extensin, expressed in grams per kilogram of total protein, c_i is the mean concentration of a unique protein-bound amino acid, i.e., 4-hydroxyproline, in grams per kilogram of total protein, n_i is the number of amino acid residues, i, per 1000 amino acid residues, WE_{P_j} is the weight equivalent of a specific protein j determined from its known amino acid composition according to the method of Horstmann (1979), and $M_{r(i)}$ is the anhydrous molecular weight of 4-hydroxyproline.

Thus, from the known distribution of 4-hydroxyproline in the primary sequence of the 30-kDa glycoprotein moiety of carrot extensin (Stuart and Varner, 1980; Van Holst and Varner, 1984; Chen and Varner, 1985a,b), which has been used as a standard for comparison in the present study (Cassab and Varner, 1988; Varner and Lin, 1989), the content of 4-hydroxyproline-rich glycoproteins of asparagus spears was calculated by multiplying the amounts of 4-hydroxyproline found in their acid hydrolysates by 2.128, as described by Khanizadeh et al. (1989).

Statistical Analysis. Data processing and statistical analysis of the results were carried out by a FORTRAN computer program developed for this purpose. Analysis of variance conducted on the amino acid data for a completely randomized block design was carried out by the general linear model procedure (SAS, 1982) on a VAX minicomputer.

RESULTS AND DISCUSSION

Analyses were carried out in the picomole range using analytical methods described previously (Zarkadas et al., 1986, 1987). The overall amino acid compositions of the selected asparagus tissues and levels of statistical significance obtained from analysis of variance are summarized in Table I. The results, expressed as grams of amino acid residues per kilogram of total protein, show deviations of less than 100 ± 2.5%. The amino acid values in Table I, expressed as grams of amino acid per kilogram of protein, show high reproducibility and low coefficients of variability. This unit for expressing the amino acid composition of a tissue was used since it reflects the relative amounts of the amino acids present and because the influence of fat, ash, and moisture are eliminated (Tristram and Smith, 1963; Eastoe, 1967). The protein content in each acid hydrolysate was determined from the summation of the weights of the amino acids present, as described by Horstmann (1979). Such calculations (eqs 1–3) yield accurate estimates of protein. Total protein on a dry weight basis for Jersey Centennial and Lucullus Mid-season averaged 23.08 and 24.40%, respectively. The constants, weight equivalent (WE, micrograms per nanomole) and conversion factors (CF and CF′, micrograms per nanomole), for each of the asparagus cultivars (Table I) or asparagus protein fractions investigated (Tables II and III) have been determined and can be used in all subsequent quantitations of these plant tissues following standard procedures as described by Horstmann (1979) and Zarkadas et al. (1988a).

The values found for total protein in asparagus (Table I) were significantly lower than the 39.4% protein value (dry weight basis) in USDA Handbook 8-11 (Haytowitz and Matthews, 1984). These values were determined using the conventional Kjeldahl nitrogen procedure. Such differences in protein content were also noted among other products evaluated by Zarkadas et al. (1988a). These data suggest that a substantial quantity of Kjeldahl nitrogen is derived from non-protein nitrogenous compounds present in asparagus and that the usefulness of the conventional Kjeldahl nitrogen conversion factor, N × 6.25 (Morries, 1983), for an accurate assessment of the total protein is limited. The best estimate protein content in asparagus appears to be a summation of the weights of amino acid residues as described by Horstmann (1979).

The amino acid compositions of asparagus spears from Jersey Centennial and Lucullus Mid-season, as presented in Table I, appeared to be very similar. Both cultivars were found to contain high levels of aspartic and glutamic acids, alanine, leucine, proline, lysine, and arginine and an overall amino acid profile which distinguished them from other plant-derived proteins. The most consistent and characteristic feature of the amino acid composition of both cultivars was the very high glutamic acid and aspartic acid contents, which together account for almost 40.3–41.0% of the total residues, compared with basic amino acids which constitute only 20% of the total. Amino acids with hydrophobic side chains, i.e., valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and tryptophan, account for a further 24.6–24.9% of the total protein. Total aromatic amino acids ranged from 35.3 to 36.7% of the total. Proline accounts for an additional 5.2–6.2%. The apparent differences noted in the levels of aspartic and glutamic acids, as well as proline and arginine, between these two cultivars were not statistically significant. There was, however, a significant variation between cultivars in the content of histidine ($P < 0.01$) and arginine ($P < 0.05$). The results of this study agree with the values in USDA Handbook 8-11 (Haytowitz and Matthews, 1984), both in terms of the amino acid composition as a whole and in many of the individual values reported.

The present study showed that asparagus spears contain other non-protein nitrogenous compounds which elute early in the chromatographic profile from tissue hydrolysates and interfere with the analysis of acidic and hydroxy amino acids including 4-hydroxyproline [Pro(4-OH)]. To overcome this difficulty, the pulverized and lyophilized asparagus spear tissues were extracted with 70% ethyl

Table I. Comparison of the Amino Acid Composition and Protein Contents of Asparagus Spears from Two Northern Adapted Cultivars

amino acid	cultivar: Jersey Centennial mean ± SEM[a]	Jersey Centennial CV[a]	cultivar: Lucullus Mid-season mean ± SEM[a]	Lucullus Mid-season CV[a]	significance levels between cultivars[a] CV	significance levels between cultivars[a] $P > F$	raw asparagus, calculated according to the method of Haytowitz and Matthews (1984)
	Grams of Amino Acids per Kilogram of Protein						
aspartic acid	184.77 ± 4.73	4.06	181.10 ± 3.01	2.88	3.76	0.58	149.93
threonine	35.25 ± 0.44	2.84	34.97 ± 1.14	5.68	3.94	0.82	35.86
serine	42.57 ● 0.66	2.68	42.61 ± 3.84	15.63	10.69	0.99	48.99
glutamic acid	203.50 ± 1.57	1.34	210.59 ± 4.03	3.31	1.92	0.16	211.58
proline	59.04 ± 1.77	5.19	49.91 ± 3.01	10.43	8.26	0.13	68.43
glycine	36.96 ± 0.21	1.00	37.64 ± 0.81	3.76	2.62	0.48	41.82
alanine	47.77 ± 1.16	4.22	47.23 ± 0.69	2.55	2.30	0.60	60.82
cyst(e)ine	13.37 ± 0.63	4.75	12.91 ± 0.13	1.79	2.29	0.20	15.21
valine	54.07 ± 0.63	2.03	53.59 ± 0.53	1.72	2.38	0.69	49.85
methionine	9.78 ± 0.34	6.09	10.45 ± 0.20	3.28	6.42	0.33	12.26
isoleucine	35.45 ± 0.62	3.06	36.03 ± 0.51	2.43	3.75	0.64	47.29
leucine	60.51 ± 0.92	2.63	61.02 ± 0.76	2.16	3.32	0.78	61.25
tyrosine	29.99 ± 0.35	2.06	30.37 ± 0.38	2.20	2.98	0.65	20.25
phenylalanine	32.44 ± 0.46	2.46	33.51 ± 0.59	3.08	3.88	0.41	30.41
histidine	20.93 ± 0.15	1.22	18.79 ± 0.23	2.19	1.99	0.02*	19.86
lysine	60.29 ± 0.22	0.64	57.95 ± 1.15	3.44	2.31	0.17	61.25
arginine	60.08 ± 1.11	3.19	67.53 ± 0.46	1.20	2.16	0.02*	60.40
tryptophan	13.23 ± 0.79	10.38	13.79 ± 0.85	10.76	10.55	0.68	12.65
ammonia	25.78 ± 1.47	9.91	25.18 ± 1.52	10.45	11.45	0.82	
total AA N	175.00 ± 1.21	1.20	175.35 ± 1.09	1.07	1.12	0.85	
g of N/kg of sample	40.06 ± 0.32	1.39	42.43 ± 0.67	2.74	2.41	0.10	
	Essential Amino Acids (EAA)						
total EAA,[b] g/g of N	2311.27 ± 30.83	2.31	2350.7 ± 33.18	2.44	3.19	0.58	
EAA_7,[c] % of total protein	27.80 ± 0.22	1.38	27.71 ± 0.39	2.46	2.72	0.89	
EAA_{10},[c] % of total protein	37.22 ± 0.34	1.60	37.72 ± 0.28	1.32	2.05	0.51	
amino acid index[d]	67.23 ± 1.02	2.62	67.41 ± 0.58	1.50	2.60	0.91	
	Calculated Protein Efficiency Ratio (PER)[d]						
predicted by[e]							
eq 5 (PER_7)	2.14 ± 0.018	1.46	2.13 ± 0.03	2.59	2.86	0.89	
eq 6 (PER_{10})	2.19 ± 0.021	1.71	2.23 ± 0.02	1.41	2.19	0.51	
	Protein Mass and Mean Residue Weight Constants						
total protein, g/kg of dry wt	230.78 ± 0.26	0.19	244.04 ± 5.22	3.71	2.82	0.13	
WE,[f] μg/nmol	0.111469 ± 0.0001	1.14	0.111927 ± 0.0002	0.29	0.25	0.18	
CF,[f] μg/nmol	0.112360 ± 0.0001	0.22	0.112863 ± 0.0002	0.39	0.33	0.24	
CF',[g] μg/nmol	0.120599 ± 0.0003	1.17	0.119822 ± 0.0007	1.05	0.81	0.43	
ethanol-soluble fraction (F1), %	62.67 ± 1.19	3.29	59.67 ± 0.17	0.48	3.29	0.22	
ethanol-insoluble fraction (F2), %	37.33 ± 1.19	5.53	40.33 ± 0.17	0.72	2.09	8.22	
moisture, %	89.43 ± 0.41	1.95	90.02 ± 2.07	9.20	7.82	0.80	

[a] Mean values and standard error of measurements (SEM) for 3 replicates and 48 determinations. Significance: $P > F$, values from analysis of variance between cultivars; **, $P < 0.01$; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [b] Computed from reference protein standards (FAO/WHO, 1965, 1973). [c] Calculated according to the method of Lee et al. (1978). EAA_7; threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. EAA_{10}: EAA_7 plus histidine, arginine, and tryptophan. [d] Calculated according to the method of Block and Mitchell (1946) and Oser (1951). [e] PER values were calculated according to the method of Lee et al. (1978) from eq 5 ($PER = 0.08084(EAA_7) - 0.1094$) and eq 6 ($PER = 0.06320(EAA_{10}) - 0.1539$). [f] The WE and CF constants were calculated according to the method of Horstmann (1979). [g] The conversion factor CF' (g) was also calculated according to eq 2 but for determining protein mass in the absence of tryptophan, cyst(e)ine, proline, and 4-hydroxyproline.

alcohol, essentially as described by Asconas (1951) and Scopes (1978). Scopes (1982) indicated that the addition of ethanol to an aqueous extract containing plant proteins has a variety of effects which in combination lead to protein precipitation. The principal effect of this addition was the reduction in dielectric constant as well as solvating power of water for a charged, hydrophilic protein molecule. On the other hand, ordered water structure around hydrophobic areas on the protein surfaces could be displaced by organic solvent molecules, leading to increased solubility of even extremely hydrophobic proteins, which are normally located in membranes. However, the net effect on cytoplasmic and other water-soluble proteins is a decrease in their solubility to the point of aggregation and precipitation. Thus, the alcohol-soluble protein fraction (F1) and alcohol-insoluble protein fraction (F2) were separated, and each of these was subjected to amino acid analysis (Zarkadas et al., 1987), so that they could form convenient standards (Table I).

The total yield obtained for the ethanol-soluble fraction (F1) ranged from 59.7 to 62.7% on a dry weight basis for Jersey Centennial and Lucullus Mid-season cultivars, respectively. The quantities of the ethanol-soluble protein fraction F1 found in the asparagus spears from Jersey Centennial and Lucullus Mid-season were 10.9–13.7% of the total protein, respectively. Lucullus Mid-season also contained a higher concentration of ethanol-insoluble protein fraction F2 (40.5%) compared to that found in the Jersey Centennial (37.2%). Since the protein concentration in each protein fraction was determined from its respective amino acid composition, the average protein yields, as presented in Tables I–III, represent accurate estimates of the amount of protein present. The average weight equivalents and conversion factors obtained from both fractions F1 and F2 are given in Tables II and III.

Although the amino acid profiles, in grams per kilogram of protein, of the ethanol-soluble protein fraction (F1) from both Jersey Centennial and Lucullus Mid-season are

Table II. Amino Acid Contents of the Ethanol-Soluble Fraction (F1) of Asparagus Spears Isolated from Two Northern Adapted Cultivars after Ethanol–Water Extraction

	cultivar				significance levels between cultivars[a]	
	Jersey Centennial		Lucullus Mid-season			
amino acid	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	CV	*F*
	Grams of Amino Acids per Kilogram of Protein					
aspartic acid	401.42 ± 11.66	5.03	374.33 ± 13.21	6.11	0.76	123.81**
threonine	15.54 ± 0.63	7.03	15.13 ± 0.43	5.26	3.68	0.81[ns]
serine	26.99 ± 2.63	14.03	24.55 ± 0.41	2.84	12.32	0.89[ns]
glutamic acid	383.50 ± 7.23	3.27	392.78 ± 8.73	3.85	3.13	0.87[ns]
proline	8.97 ± 1.51	20.18	11.04 ± 1.40	21.96	13.59	3.50[ns]
glycine	18.29 ± 1.07	10.15	15.03 ± 0.68	7.83	11.25	4.56[ns]
alanine	31.58 ± 3.41	18.69	29.09 ± 2.46	4.65	3.91	6.61[ns]
valine	27.65 ± 2.17	13.62	29.69 ± 2.11	12.30	0.91	89.33**
methionine	5.04 ± 0.53	5.19	5.23 ± 1.24	41.01	41.38	0.01[ns]
isoleucine	2.91 ± 0.49	28.63	3.98 ± 0.05	2.23	16.86	5.08[ns]
leucine	7.24 ± 0.15	3.69	8.69 ± 0.36	7.09	7.65	11.57[ns]
tyrosine	0.00 ± 0.00	0.00	1.29 ± 0.10	13.610	19.25	161.86**
phenylalanine	0.00 ± 0.00	0.00	1.70 ± 0.11	1.74	15.18	260.04**
histidine	16.25 ± 0.62	30.62	14.96 ± 0.52	5.99	24.77	0.17[ns]
lysine	16.72 ± 0.74	7.69	18.72 ± 0.59	5.53	0.99	193.09**
arginine	39.32 ± 2.68	11.84	54.05 ± 0.81	2.59	6.78	32.38*
4-hydroxyproline	1.23 ± 0.06	9.11	1.46 ± 0.07	9.24	10.93	3.89[ns]
ammonia	36.98 ± 0.87	4.07	32.09 ± 2.58	13.95	12.22	2.01[ns]
	Protein Mass, Nitrogen, and Mean Residue Weight Constants[b]					
total AA N	169.42 ± 1.99	2.03	165.68 ± 1.49	1.55	2.52	1.17[ns]
g of N/kg of dry wt	17.69 ± 0.23	2.21	22.75 ± 1.25	9.48	8.33	13.51[ns]
g of total AA/16 g of N	94.47 ± 1.12	2.05	96.59 ± 0.86	1.54	2.52	1.16[ns]
total protein,[b] g/kg of dry wt	109.62 ± 4.43	7.01	137.39 ± 8.03	10.12	11.72	5.51[ns]
WE,[b] μg/nmol	0.11515 ± 0.0005	0.74	0.11650 ± 0.00009	0.13	0.45	3.89[ns]
CF,[b] μg/nmol	0.11664 ± 0.0003	0.49	0.11830 ± 0.00014	0.19	0.31	29.28*
	Essential Amino Acids (EAA)					
total EAA,[c] g/g of N	709.69 ± 3.85	0.93	839.29 ± 27.63	5.70	4.55	20.27*
EAA index[d]	11.01 ± 0.05	0.81	13.12 ± 0.36	0.35	4.73	39.72*
EAA_7,[e] % of total protein	8.03 ± 0.06	1.28	8.30 ± 0.41	8.61	6.59	0.38[ns]
EAA_{10},[e] % of total protein	13.83 ± 0.44	5.59	15.04 ± 0.50	5.74	5.37	3.63[ns]
	Calculated Protein Efficiency Ratio (PER)[e]					
predicted by[e]						
eq 5 (PER^7)	0.52 ± 0.02	6.55	0.56 ± 0.03	10.29	10.16	0.67[ns]
eq 6 (PER^{10})	0.72 ± 0.02	6.79	0.79 ± 0.032	7.05	6.08	3.68[ns]

[a] Mean values and standard error of measurements (SEM) for 3 replicates and 48 determinations. Significance: *F*, values from analysis of variance between treatments; **, $P < 0.01$; *, $P < 0.05$; ns, not significant, CV, coefficient of variation. [b] The total protein, WE, and CF constants (μg/nmol) were calculated according to the method of Horstmann (1979), where WE is the mean residue weight and CF is the apparent average molecular weight increased in proportion to the missing tryptophan and cyst(e)ine values. [c] Computed from reference protein standards (FAO/WHO, 1973). [d] Calculated according to the methods of Block and Mitchell (1946) and Oser (1951). [e] Calculated according to the method of Lee et al. (1978). EAA_7: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. EAA_{10}: EAA_7 plus histidine, arginine, and tryptophan. PER (protein efficiency ratio) values were calculated according to the method of Lee et al. (1978) from eq 5 (PER = 0.08084 (EAA_7)) and eq 6 (PER = 0.06320(EAA_1) − 0.1539).

very similar, significant differences ($P < 0.01$) were noted in their contents of aspartic acid, valine, lysine, and arginine. Glutamic and aspartic acids are the most abundant amino acids and, when taken together, account for almost 76.7–78.5% of all residues in the ethanol-soluble protein fraction. The present mean value for basic amino acids ranged from 7.2 to 8.8% of the total amino acids. The aromatic amino acids tyrosine and phenylalanine were present in very low concentration in Lucullus Mid-season and were absent in Jersey Centennial. It should be noted that 4-hydroxyproline was also found at very low levels in both cultivars.

A summary of the amino acid profiles of the ethanol-insoluble protein fraction extracted from Jersey Centennial and Lucullus Mid-season, as presented in Table III, shows that they are similar in composition. Both fractions were found to contain high levels of aspartic and glutamic acids, proline, leucine, lysine, and arginine and overall amino acid profiles which distinguished them from other plant tissues. Glutamic acid accounted for 12.7% of amino acids in the Lucullus Mid-season and 12.8% in Jersey Centennial, respectively. Aspartic acid, the next most abundant amino acid, accounted for a further 10.0%. The mean values for total basic amino acids were similar for both cultivars and ranged from 18.0 to 18.3%. The differences noted for 4-hydroxyproline and arginine were significant at the 5% level.

From the results presented in Table III, it is apparent that the ethanol-insoluble protein fraction (F2) contained protein-bound 4-hydroxyproline, Lucullus Mid-season being consistently higher in Pro(4-OH) than Jersey Centennial. Mean values in the ethanol-insoluble fraction F2 were 3.1 g/kg of total protein for Jersey Centennial and 4.9 g/kg for Lucullus Mid-season. Small amounts of free Pro(4-OH) were present in the ethanol-soluble fraction, F1 (Table II). Pro(4-OH) was once thought to be unique to collagen and elastin (Eastoe, 1967). However, recent evidence has suggested that this hydroxylated unique amino acid is a structural component of the 4-hydroxyproline-rich glycoproteins found in the extracellular matrices of primary cell wall of plants (angiosperm) (Lamport, 1980; Fincher et al., 1983; McNeil et al., 1984; Cooper et al., 1987; Cassab and Varner, 1988) and seeds including the cell walls of soybean seed coats (Cassab et al., 1985; Cassab and Varner, 1987, 1988), developing soybean tissue (Ye and Varner, 1991), and the cell walls of wounded and

Table III. Comparison of the Amino Acid Composition and Protein Contents of Ethanol-Insoluble Fractions (F2) Isolated from Asparagus Spears from Two Northern Adapted Cultivars after Ethanol–Water Extraction

	cultivar				significance levels between cultivars[a]	
	Jersey Centennial		Lucullus Mid-season			
amino acid	mean ± SEM[a]	CV[a]	mean ± SEM[a]	CV[a]	CV[a]	$P > F^a$
	Grams of Amino Acids per Kilogram of Protein					
aspartic acid	97.94 ± 0.14	0.24	97.40 ± 0.81	1.44	1.17	0.62
threonine	41.89 ± 0.05	0.22	41.64 ± 0.48	2.02	1.42	0.65
serine	45.12 ± 0.88	3.38	45.44 ± 1.88	7.17	5.67	0.89
glutamic acid	125.56 ± 0.49	0.68	123.51 ± 0.21	0.31	0.70	0.10
proline	58.05 ± 0.88	2.63	58.55 ± 2.18	6.45	5.74	0.87
glycine	47.47 ± 0.24	0.87	46.53 ± 0.52	1.90	1.95	0.94
alanine	56.11 ± 0.22	0.68	55.51 ± 0.18	0.56	0.61	0.16
cyst(e)ine	32.15 ± 0.48	2.61	31.24 ± 0.47	2.60	1.92	0.21
valine	64.99 ± 0.39	1.03	65.02 ± 0.39	1.06	0.61	0.93
methionine	15.21 ± 0.18	2.13	16.07 ± 0.37	4.02	2.79	0.13
isoleucine	51.13 ± 0.21	0.72	50.97 ± 0.23	0.80	1.07	0.76
leucine	85.79 ± 0.41	0.82	85.61 ± 0.34	0.71	1.02	0.82
tyrosine	41.28 ± 0.52	2.19	41.29 ± 0.15	0.63	1.15	0.89
phenylalanine	48.17 ± 0.59	2.13	48.16 ± 0.47	1.70	2.71	0.99
histidine	27.07 ± 0.48	3.07	26.98 ± 0.21	1.32	1.72	0.82
lysine	75.50 ± 0.24	0.55	75.82 ± 0.21	0.47	0.72	0.54
arginine	70.20 ± 0.19	0.46	72.01 ± 0.44	1.05	0.54	0.02*
tryptophan	13.39 ± 1.66	21.48	12.78 ± 0.76	10.33	9.39	0.38
4-hydroxyproline	2.97 ± 0.41	23.63	4.53 ± 0.63	24.11	8.29	0.02*
ammonia	9.63 ± 0.61	11.09	10.05 ± 0.17	2.88	6.59	0.51
	Protein Mass, Nitrogen, and Mean Residue Weight Constants[b]					
total AA N	168.64 ± 1.93	5.20	169.43 ± 0.91	2.38	3.77	0.41
g of AA/16 g of N	94.87 ± 0.26	0.48	94.44 ± 0.03	0.07	0.34	0.23
g of N/kg of dry wt	64.08 ± 1.92	5.20	66.17 ± 0.91	2.39	1.83	0.12
total protein, g/kg of dry wt	382.98 ± 10.50	4.74	393.56 ± 5.68	2.50	3.35	0.42
WE, μg/nmol	0.11037 ± 0.0001	0.19	0.11039 ± 0.0001	0.10	0.178	0.88
CF, μg/nmol	0.11126 ± 0.0002	0.66	0.11123 ± 0.0004	0.12	0.30	0.62
CF′, μg/nmol	0.11956 ± 0.0001	0.20	0.11981 ± 0.0003	0.44	0.45	0.42
	Essential Amino Acids (EAA)[e]					
total EAA,[e] g/g of N	3200.3 ± 15.58	0.84	3190.4 ± 10.9	0.59	0.24	0.25
EAA/ index	92.97 ± 1.28	2.39	92.24 ± 0.49	0.92	2.11	0.69
EAA_7,[e] % of total protein	36.74 ± 0.11	0.51	36.72 ± 0.11	0.50	0.69	0.91
EAA_{10},[g] % of total protein	47.81 ± 0.14	0.52	47.90 ± 0.15	0.53	0.57	0.73
	Calculated Protein Efficiency Ratio (PER)					
predicted by						
eq 5 (PER^7)	2.86 ± 0.0008	0.52	2.85 ± 0.01	0.51	0.72	0.91
eq 6 (PER^{10})	2.87 ± 0.0009	0.55	2.87 ± 0.01	0.56	0.60	0.41

[a] Mean values and standard error of measurements (SEM) for three replicates and 48 determinations. Significance: F, values from analysis of variance between treatments; *, $P < 0.05$; ns, not significant; CV, coefficient of variation. [b] Calculated according to the method of Heidelbaugh et al. (1975). [c] The total protein, WE, and CF constants were calculated according to the method of Horstmann (1979), where CF is the apparent average residue molecular weight increased in proportion to the missing tryptophan and cyst(e)ine values. Dry wt, dry weight basis. [d] Data for 4-hydroxyproline-rich glycoproteins were calculated from the amount of 4-hydroxyproline found in the acid hydrolysates of asparagus spears according to eq 4, as described by Khanizadeh et al. (1989), and represent the mean values for 12 determinations from 3 replicates. [e] Computed from reference protein standards (FAO/WHO, 1965, 1973). [f] Calculated according to the method of Block and Mitchell (1946) and Oser (1951). [g] Calculated according to the method of Lee et al. (1978). EAA_7: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. EAA_{10}: EAA_7 plus histidine, arginine, and tryptophan. [h] PER (protein efficiency ratio) values were calculated according to the method of Lee et al. (1978) from eq 5 ($PER = 0.08084(EAA_7) - 0.1094$) and eq 6 ($PER = 0.06320(EAA_{10}) - 0.1539$).

Table IV. Comparison of the Essential Amino Acid (EAA) Composition of Two Northern Adapted Asparagus Cultivars and Raw Asparagus with the Suggested EAA Requirements for Humans

		EAA composition		
EAA	EAA requirements for preschool child (2–5 years)[a]	Jersey Centennial	Lucullus Mid-season	raw asparagus, (Haytowitz and Matthew, 1984)
histidine	19	20.9	18.8	19.9
isoleucine	28	35.5	36.0	47.3
leucine	66	60.5	61.0	61.3
lysine	58	60.3	58.0	61.3
methionine + cyst(e)ine	25	23.2	23.4	27.5
phenylalanine + tyrosine	63	62.4	63.9	50.7
threonine	34	35.3	35.0	35.9
tryptophan	11	13.2	13.8	12.7
valine	35	54.0	53.6	49.9
total	339	365.1	362.9	366.2

[a] Data from FAO/WHO/UNU (1985).

infected plants (Corbin et al., 1987). The 86-kDa carrot extensin monomer is the only one that has been characterized; it consists of 35% protein and 65% carbohydrate (Stuart and Varner, 1980; Van Holst and Varner, 1984). The 30-kDa protein moiety contains 306 amino acids in its primary sequence (Chen and Varner, 1985a,b; Showalter

and Rumeau, 1990; Smith et al., 1986; Kielszewski et al., 1992), and Pro(4-OH) makes up 45.5% of the polypeptide backbone, and it has been used as a standard for comparison in the present study (Varner and Lin, 1989; Ye and Varner, 1991; Marcus et al., 1991). The content of 4-hydroxyproline-rich glycoproteins of asparagus was calculated by multiplying the amounts of Pro(4-OH) found in their acid hydrolysates by 2.128 (eq 4), as described by Khanizadeh et al. (1989). The values of 4-hydroxyproline-rich glycoproteins of 6.32 and 9.64 g of glycoprotein/kg of total protein for Jersey Centennial and Lucullus Mid-season, respectively, were considerably higher than those reported by Khanizadeh et al. (1989) for apple flower buds. Similar studies on asparagus have not been previously reported.

A comparison of the essential amino acid (EAA) profiles of asparagus spears from Jersey Centennial and Lucullus Mid-season according to the procedures of Block and Mitchell (1946), Oser (1951), Lee et al. (1978), McLaughlan et al. (1980), Pellett and Young (1984, 1988), Sarwar (1984), FAO/WHO/UNU (1985), Young (1987), Young et al. (1989), Millward et al. (1989), and FAO/WHO (1990) indicates that asparagus contained all essential amino acids (EAA) required for human nutrition but was slightly limiting in methionine, cyst(e)ine, and leucine. Mean values for total EAA_7 and EAA_{10} ranged, respectively, from 28.6 to 37.6% and from 43.2 to 47.3% in the asparagus spears evaluated (Tables I–III). Because this scoring procedure is by definition limited to essential amino acids, Lee et al. (1978) developed equations (eqs 5 and 6 listed in Tables I–III) for predicting protein efficiency ratios (PERs) (Expert Work Group, 1984). In using these predictive equations, the results summarized in Tables I–III showed that PER values for the two cultivars were 2.1 and 2.8. In this study the protein ratings of asparagus spears were calculated according to the method recommended by FAO/WHO (1990) on protein quality evaluation of foods, and the results obtained (Table IV) were compared with those of the reference pattern for preschool children (2–5 years) established by FAO/WHO/UNU (1985). Pellett and Young (1988) and Young et al. (1989) have proposed that this amino acid scoring method (preschool-aged group), as recommended by FAO/WHO/UNU (1985), be employed for all ages except infants. The results presented in Table IV indicate (1) that the asparagus proteins of the new cultivars, although small in amount, contain all of the essential amino acids with the exception of the observed lower level of sulfur-containing amino acids and possibly leucine and (2) that a potentially useful means for evaluating the protein quality of perennial crops might be based on a knowledge of their amino acid composition as recommended by FAO/WHO/UNU (1985) and FAO/WHO (1990).

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Received for review April 16, 1992. Accepted September 14, 1992.