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Amino Acid Concentrations and Comparison of Different Hydrolysis Procedures for American and Foreign Chestnuts

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Amino acid concentrations were determined in chestnut meats of the American chestnut (*Castanea dentata*), Chinese chestnut (*Castanea molissima*), an American hybrid chestnut grown in the United States, and a European chestnut (*Castanea sativa*) grown in Italy. A comparison is made of two hydrolysis methods using sealed tube and reflux hydrolysis procedures. Performic acid oxidation with acid hydrolysis was used for liberation of the sulfur amino acids. Alkaline hydrolysis was used to free tryptophan. Chestnuts, which have high carbohydrate levels, gave low amino acid recoveries. Slightly higher amino acid recoveries were obtained from the reflux procedure. Performic acid oxidation produced greater recoveries of the sulfur amino acids than either the sealed tube or reflux hydrolysis procedure.

Chestnuts have been used for food and timber since ancient times in northern China (Payne et al., 1983). The American chestnut (*Castanea dentata*) was once the most important hardwood species in the Eastern United States. However, the chestnut blight in the early 1900s caused the destruction, in less than 40 years, of every major stand of American chestnut (Anagnostakis, 1978). Currently, less than 160 ha of commercial chestnut orchards are in existence in the United States (Payne et al., 1983). In the United States the lack of availability of chestnut meats has limited their use as a food. Chinese chestnuts (*Castanea molissima*), which are blight resistant, are now being sold in place of the American chestnut for orchards and the home grower (Jaynes, 1979). As chestnuts from these new plantings become available, this nut should achieve wider acceptance from the American consumer.

Amino acid data have been published on the Japanese chestnut by Taira and Taira (1964), Manabe (1975), and the Food and Agricultural Organization (1972) and on the European chestnut by Souci et al. (1981). Data on the amino acid composition of American chestnuts are not available.

This paper compares the amino acid and ammonia concentrations obtained from two hydrolysis procedures of three chestnut species *C. denta*, *C. molissima*, and

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Table I. Total Nitrogen,[a] Amino Acids,[b] Ammonia,[b] Total Amino Acids and Ammonia,[b] and the Percent Amino Acid Plus Ammonia Recovered from Chestnuts Hydrolyzed[c] by the Sealed Tube Procedure[d]

	hybrid, IL	American, IL	Italian	Chinese	American, PA
total N	1.32 ± 0.01	1.51 ± 0.03	0.83 ± 0.02	1.42 ± 0.03	1.62 ± 0.02
Asp	10.63 ± 0.16	10.53 ± 0.25	14.13 ± 0.36	17.22 ± 0.26	14.76 ± 0.19
Thr	3.51 ± 0.06	3.29 ± 0.08	2.92 ± 0.02	3.13 ± 0.03	2.92 ± 0.01
Ser	3.65 ± 0.08	3.49 ± 0.07	3.75 ± 0.11	3.34 ± 0.20	3.07 ± 0.04
Glu	12.21 ± 0.22	14.63 ± 0.54	10.40 ± 0.23	10.84 ± 0.20	10.92 ± 0.19
Pro	5.50 ± 0.14	4.14 ± 0.23	4.09 ± 0.08	3.26 ± 0.07	3.60 ± 0.05
Gly	4.44 ± 0.07	4.36 ± 0.11	3.91 ± 0.12	3.73 ± 0.05	3.99 ± 0.12
Ala	5.20 ± 0.05	4.48 ± 0.06	5.45 ± 0.08	4.01 ± 0.07	4.31 ± 0.04
Val	5.15 ± 0.06	4.57 ± 0.08	4.61 ± 0.17	4.39 ± 0.04	4.66 ± 0.06
Met	1.11 ± 0.03	0.95 ± 0.02	0.89 ± 0.07	0.69 ± 0.18	0.67 ± 0.04
Ile	3.82 ± 0.06	3.54 ± 0.10	3.05 ± 0.03	3.08 ± 0.01	3.25 ± 0.03
Leu	6.33 ± 0.09	6.12 ± 0.20	4.82 ± 0.23	5.01 ± 0.06	5.41 ± 0.05
Tyr	2.59 ± 0.06	2.74 ± 0.11	1.99 ± 0.04	1.67 ± 0.49	1.57 ± 0.03
Phe	3.75 ± 0.07	3.81 ± 0.08	3.40 ± 0.02	3.32 ± 0.07	3.30 ± 0.03
His	2.54 ± 0.05	2.17 ± 0.02	2.24 ± 0.08	2.31 ± 0.07	2.23 ± 0.57
Lys	5.53 ± 0.09	4.75 ± 0.04	4.65 ± 0.17	4.57 ± 0.12	4.73 ± 0.05
Arg	6.32 ± 0.05	8.90 ± 0.18	5.94 ± 0.16	7.80 ± 0.54	5.98 ± 0.10
NH_4^+	1.30 ± 0.05	1.26 ± 0.09	1.45 ± 0.02	1.56 ± 0.01	1.32 ± 0.04
total AA + NH_4^+[e]	83.58[z][f]	83.73[z]	77.69[x]	79.93[y]	76.69[x]
% rec[g]	74.2	77.3	69.5	72.9	68.4

[a] Grams per 100 g. [b] Grams of amino acid/16 g of nitrogen. [c] Based on 24-h hydrolysis time. [d] Uncorrected data. [e] Total amino acids plus NH_4^+ (g of AA/16 g of N). [f] Mean separation by Duncan's multiple-range test, 5%. [g] Individual amino acids plus ammonia.

Castanea sativa and two samples of American chestnut *C. denta*. Comparisons of the above two acid hydrolysis procedures and performic acid hydrolysis are reported for concentrations of the sulfur amino acids. Tryptophan levels, obtained by alkaline hydrolysis, are presented for the chestnuts.

MATERIAL AND METHODS

Chemicals and Reagents. Chemicals and standards used in the amino acid analysis were purchased from Pierce Chemical Co., Rockford, IL.

Plant Material. Three chestnut species from four different locations were used in the investigation. Two of the chestnut samples, an American hybrid and the American chestnut (*C. dentata*), were obtained from a commercial nursery at O'Sallon, IL. The American chestnut (*C. dentata*) was purchased from a commercial nut buyer at Williams Port, PA. Dr. Richard Jaynes, Connecticut Agricultural Experiment Station, New Haven, CT, supplied the Chinese chestnuts (*C. molissima*). The remaining chestnut sample (*C. sativa*) was purchased from a commercial importer who obtained it from Italy.

On arrival at the laboratory, the chestnuts were shelled, freeze-dried, ground to pass a 40-mesh screen, ball-milled for 24 h, and stored under vacuum over phosphorus pentoxide until chemically analyzed.

Total Nitrogen. Kjeldahl nitrogen was determined in duplicate by the micro-Kjeldahl method (AOAC, 1980).

Amino Acid Hydrolysis. The amino acid analyses were carried out in duplicate and were replicated three times. A 1-g sample of finely ground dry chestnut flour was refluxed in 250 mL of 6 N HCl for 24 h. The HCl was removed from the hydrolyzed sample on a rotary evaporator, brought to a known volume with pH 2.2 citrate buffer, filtered through a 0.45-μm microporous filter (HVLP 02500, Millipore Corp., Bedford, MA), and stored at −30.5 °C prior to amino acid analysis. Twenty milligrams of chestnut flour was hydrolyzed for 24 h by the modified (Meredith et al., 1986) sealed tube procedure of Moore and Stein (1963). The alkaline hydrolysis method of Hugli and Moore (1972) was used to prepare each of the chestnut samples for the determination of tryptophan. Immediately after alkaline hydrolysis the sample was analyzed on the amino acid analyzer. To convert cystine and cysteine to cysteic acid and methionine to methionine sulfate, each of the chestnut samples was subjected to an overnight performic acid oxidation procedure and acid hydrolysis (Moore, 1963).

Amino Acid Analysis. Amino acids were determined on a Durrum D501 automatic amino acid analyzer equipped with a Mark II data processor (Durrum Instrument Corp., Palo Alto, CA) using ninhydrin as the color reactant and a single ion-exchange column (0.175 cm × 48 cm) for separation. Column temperature and buffers used in the standard amino acid separation are described in the Durrum amino acid manual (1972). A standard, six samples, and a standard were the order in which samples were analyzed on the analyzer.

Buffers used in the separation of tryptophan were sodium citrate, pH 6.50 ± 0.01 (0.20 N) and pH 7.90 (1.10 N) (Dixon, P., personal communication). Column temperature and flow rate was the same as above.

Cysteic acid and methionine sulfone were separated by the same analytical conditions used to separate the 16 amino acids above.

Statistical Analysis. The data were analyzed by the ANOVA program of the Statistical Analysis System (SAS) (Ray, 1982; Little and Hills, 1978).

RESULTS AND DISCUSSION

The Kjeldahl nitrogen (total N) content, data for 16 amino acids and ammonia, the standard deviations, and the percent amino acids recovered from the five chestnut samples hydrolyzed by the sealed tube method are presented in Table I. Kjeldahl nitrogen in the chestnuts ranged from 0.83 g of N/100 g dry weight to 1.62 g of N/100 g dry weight. Concentrations (grams of AA/16 g of N) for the 16 amino acids were very similar among the five chestnut samples. Chestnuts grown in Pennsylvania (PA) had the lowest total amino acids plus ammonia (76.69 g of AA/16 g of N) while the highest total amino acids plus ammonia (83 g of AA/16 g of N) were found in the hybrid and American varieties grown in Illinois (IL). Recovery values for the amino acids ranged from 68% to 77%. Determination of total carbohydrate concentrations by difference [(100 − (protein + lipid + ash) = total carbohydrate concentration), protein = total N × 5.30] showed that American PA contained 86.5 g/100 g dry weight, Chinese chestnut contained 87.9 g/100 g dry weight, and Italian contained 90.1 g/100 g dry weight (McCarthy and Meredith, 1988). The high carbohydrate level in the chestnuts is capable of reacting with liberated amino acids

Table II. Amino Acids and Ammonia,[a] Total Amino Acids Plus Ammonia,[a] and the Percent Amino Acid and Ammonia Recovered from Chestnut Hydrolyzed[b] by the Reflux Procedure[c]

	hybrid, IL	American, IL	Italian	Chinese	American, PA
Asp	10.97 ± 0.20	10.90 ± 0.28	14.47 ± 0.23	17.22 ± 0.62	14.18 ± 0.40
Thr	3.69 ± 0.08	3.45 ± 0.10	3.11 ± 0.12	3.26 ± 0.06	3.01 ± 0.03
Ser	3.96 ± 0.09	3.73 ± 0.17	4.26 ± 0.45	3.44 ± 0.04	3.43 ± 0.05
Glu	12.53 ± 0.32	13.97 ± 0.18	10.95 ± 0.32	10.88 ± 0.10	10.91 ± 0.16
Pro	5.60 ± 0.11	4.25 ± 0.26	4.00 ± 0.21	3.03 ± 1.89	3.46 ± 0.15
Gly	4.53 ± 0.10	4.35 ± 0.21	4.03 ± 0.23	3.71 ± 0.03	3.98 ± 0.07
Ala	5.14 ± 0.12	4.38 ± 0.14	5.94 ± 0.24	4.04 ± 0.18	4.33 ± 0.10
Val	4.73 ± 0.15	3.94 ± 0.08	4.17 ± 0.11	3.91 ± 0.06	3.98 ± 0.08
Met	0.74 ± 0.13	0.68 ± 0.07	0.77 ± 0.15	0.55 ± 0.02	0.64 ± 0.02
Ile	3.72 ± 0.08	3.42 ± 0.12	3.04 ± 0.11	2.99 ± 0.01	3.08 ± 0.05
Leu	6.28 ± 0.17	5.92 ± 0.15	4.90 ± 0.17	5.46 ± 0.46	5.27 ± 0.07
Tyr	3.24 ± 0.08	3.42 ± 0.24	2.15 ± 0.08	2.84 ± 0.42	2.51 ± 0.10
Phe	3.72 ± 0.06	3.78 ± 0.14	3.31 ± 0.16	3.92 ± 0.02	3.31 ± 0.03
His	2.39 ± 0.06	2.16 ± 0.08	2.23 ± 0.14	2.55 ± 0.36	2.17 ± 0.01
Lys	5.47 ± 0.14	4.79 ± 0.16	4.82 ± 0.14	4.69 ± 0.12	4.72 ± 0.06
Arg	6.51 ± 0.20	9.14 ± 0.34	6.20 ± 0.12	8.94 ± 0.05	6.71 ± 0.12
NH_4^+	1.30 ± 0.05	1.48 ± 0.08	1.84 ± 0.08	2.08 ± 0.29	1.50 ± 0.09
total AA + NH_4^+[d]	84.25[z][e]	83.76[z]	80.19[y]	83.51[z]	77.19[x]
% rec[f]	75.9	77.8	72.1	77.6	70.0

[a] Grams of amino acid per 16 g of nitrogen. [b] Based on 24-h hydrolysis time. [c] Uncorrected data. [d] Total amino acids plus ammonia. [e] Mean separation by Duncan's multiple-range test, 5% level. [f] Individual amino acids plus ammonia.

Table III. Significance[a] between the Sealed Tube and Reflux Hydrolysis Procedures

	signif		signif
Asp	*	Ala	*
Thr	***	Val	***
Ser	***	Met	**
Glu	NS	Ile	**
Pro	*	Leu	NS
Gly	NS		
Tyr	***	Phe	***
		His	*
		Lys	*
		Agr	***
		NH_4^+	***
		% rec	**

[a] Statistically significant at the 5% (*), 1% (**), and 0.1% (***) levels and nonsignificant (NS).

during hydrolysis (Maillard browning reaction), thus producing low amino acid recoveries (Koehler et al., 1969; Springarn et al., 1983; Ashoor and Zent, 1984).

The five chestnut samples hydrolyzed by the reflux procedure are presented in Table II. The amino acid concentrations were generally slightly higher for the reflux procedure than with the sealed tube methods. This is reflected in the higher total amino acids plus ammonia and in the higher percentage recovery values obtained with the reflux hydrolysis procedure.

Analyses of variance between samples using the sealed tube and the reflux procedure are given in Table III. Differences in glutamic acid, glycine, and leucine were not significant with respect to the two hydrolysis procedures used; however, the remaining amino acid concentrations were significant at $P < 0.05$, $P < 0.01$, or $P < 0.001$ probability. Wolfrom et al. (1974) reported that in model systems of D-glucose and L-arginine solutions that as arginine content was decreased Maillard browning decreased and of the amino acids studied only 4-aminobutyric acid suppressed arginine in promoting the Maillard browning reaction. A highly significant difference ($P < 0.001$) between the two hyrolysis procedures (Table III) was found for arginine. Other amino acids that were highly significant ($P < 0.001$) were aspartic acid, serine, alanine, leucine, and tyrosine and the nonamino acid ammonia.

Cysteic acid and methionine sulfone concentrations (Table IV) determined by the performic acid oxidation and then acid hydrolysis by the sealed tube procedure gave consistent values as evidenced by the small standard deviation. The performic acid oxidation and acid hydrolysis procedure greatly increased the accuracy of the determination of the sulfur amino acids as Moore (1963) demonstrated recoveries of 94 ± 2% for cysteic acid and Blackburn (1968) reported 100 ± 2% for methionine sulfone. Our values for the determination of cystine by only acid hydrolysis using the sealed tube or reflux procedure were very erratic, with concentrations ranging from 0.0 to 0.02 g of AA/16 g of N. Their values were not reported. Tryptophan concentration in the chestnuts was lowest in the American PA (0.80 g of AA/16 g of N) and highest in the American IL (1.27 g of AA/16 g of N) (Table IV).

CONCLUSIONS

Amino acid composition data are presented for five chestnut varieties grown in the United States and Italy. Amino acid concentrations differed only slightly among the five chestnut samples. The essential sulfur amino acid methionine was found to be highest in the Italian chestnut and lowest in the American PA chestnut. Slightly higher amino acid concentration values were determined by using the reflux procedure over the sealed tube procedure for the hydrolysis. One disadvantage of the reflux procedure in our laboratory was that large numbers of samples could not be hydrolyzed in a single day (three samples in duplicate) whereas eight samples could be hydrolyzed in duplicate by the sealed tube procedure. Therefore, the slightly higher amino acid concentrations obtained from

Table IV. Cysteic Acid, Methionine Sulfone, and Tryptophan Concentrations[a] in Chestnuts

	hybrid, IL	American, IL	Italian	Chinese	American, PA
Cys A[b]	2.28[zy][c] ± 0.04	2.35[z] ± 0.03	2.69[x] ± 0.03	2.22[y] ± 0.04	1.88[w] ± 0.03
Met S[b]	1.77[z] ± 0.02	1.69[z] ± 0.03	2.01[y] ± 0.02	2.04[y] ± 0.03	1.56[x] ± 0.02
Trp[d]	1.00[z] ± 0.01	1.27[y] ± 0.01	0.94[x] ± 0.01	0.99[z] ± 0.01	0.80[w] ± 0.02

[a] Grams of amino acid per 16 g of nitrogen. [b] Performic acid oxidation and acid hydrolysis. [c] Mean separation by Duncan's multiple-range test, 5% level. [d] Alkaline hydrolysis.

the reflux hydrolysis procedure may not justify the additional time required to hydrolyze a set of samples compared with the sealed tube hydrolysis procedure, especially if the set of samples being analyzed contain low concentrations of carbohydrate. Performic acid oxidation and acid hydrolysis by the sealed tube procedure greatly increases the accuracy of the sulfur amino acid data and is the only method for obtaining reliable sulfur amino acid data in samples containing high carbohydrate concentrations.

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Registry No. Asp, 56-84-8; Thr, 72-19-5; Ser, 56-45-1; Glu, 56-86-0; Pro, 147-85-3; Gly, 56-40-6; Ala, 56-41-7; Val, 72-18-4; Met, 63-68-3; Ile, 73-32-5; Leu, 61-90-5; Tyr, 60-18-4; Phe, 63-91-2; His, 71-00-1; Lys, 56-87-1; Arg, 74-79-3; N_2, 7727-37-9; NH_4^+, 7664-41-7.

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