Changes in Composition of the Nonprotein-Nitrogen Fraction of "Jewel" Sweet Potatoes (*Ipomoea batatas* (Lam.)) during Storage

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The composition of the nonprotein-nitrogen (NPN) fraction of "Jewel" sweet potatoes was studied during 281 days of storage. The major components of the NPN at 107 days were asparagine (61%), aspartic acid (11%), glutamic acid (4%), serine (4%), and threonine (3%). Levels of most of the amino acids changed with time; regression equations are given.

Under some conditions, sweet potatoes may provide a significant amount of protein to the human diet (Adolph and Liu, 1939). In "Jewel" sweet potatoes, as much as 40% of the total nitrogen may be nonprotein nitrogen (Purcell et al., 1978a). Nonprotein nitrogen (NPN) is defined as the nitrogen contained in compounds soluble in 12% trichloroacetic acid (Mezincescu and Abo, 1936). Such a classification would include peptides too small to be precipitated, free amino acids, amides, and other nonpolymeric nitrogen compounds. If the NPN contains large amounts of non amino acid nitrogen, the large amount of NPN could alter the estimated nutritional value of sweet potatoes. In previous work, sweet potatoes were fractionated biochemically, and the amino acid composition of each fraction was determined (Purcell et al., 1978b). The syrup fraction contained large amounts of aspartic acid, glutamic acid, and ammonia. Nitrogen in the syrup was not heat precipitable and was suspected to be similar to NPN. Purcell et al. (1978a) reported that during storage the percentage of NPN in sweet potatoes declined to a minimum and then increased, suggesting active nitrogen metabolism. We studied the composition of the NPN of "Jewel" sweet potatoes to determine whether it changed during 281 days of storage. Results of that study are reported here.

MATERIALS AND METHODS

Sweet Potatoes. "Jewel" sweet potatoes were grown at the North Carolina Agricultural Research Service, Central Crops Research Station near Clayton, NC, following standard cultural practices (Covington et al., 1976). The roots were harvested on Sept 20, 1977, and were cured for 5 days at 30 °C and 90–95% relative humidity. After curing, the roots were stored at 13 °C without humidity control. On June 1, 1978, the temperature was increased to 25 °C to simulate conditions for preplant sprouting.

At each of 22 sampling periods, one root was randomly selected from each of six boxes set aside for this study.

Compositional Analyses of NPN. A homogenate was prepared from 50 g of fresh slices and 150 mL of 13% trichloroacetic acid (Cl_3CCOOH) in H_2O, transferred to centrifuge tubes, using another 50 mL of 13% Cl_3CCOOH, and centrifuged at 10000*g* for 10 min (Figure 1). The supernatant was decanted and filtered through a Whatman no. 1 filter paper. Nitrogen content of the extract was determined by Kjeldahl analysis. Four 4-mL aliquots were taken to dryness at 40 °C in a rotary film evaporator. Two samples (nonhydrolyzed) were taken up in sample dilution buffer (Spackman et al., 1958) for amino acid analysis. The other two samples were transferred to 10-mL ampules with 2 mL of 12 N HCl, 2 mL of H_2O, and 4 mL of glacial propionic acid. Necks of the ampules were drawn to an inside diameter of about 1 mm. The ampules were placed into a desiccator, which was then evacuated until bubbles formed in the samples. Vacuum was released with nitrogen. This procedure was repeated four times. The ampules were removed from the desiccator and connected to a vacuum source set at 0.5 atm, the necks were sealed, and the ampoules were heated to 121 °C for 2 h. The hydrolyzed samples were dried as before, taken up in a 5-mL sample of dilution buffer for amino acid analysis, and filtered through a 0.2-μm Millipore filter.

In the later stages of this work, 50-g samples of fresh slices were extracted with 70% aqueous MeOH, and soluble nitrogen was determined as NPN. These samples were analyzed the same way that the Cl_3CCOOH extracts were.

Amino Acid Analysis. An aliquot (0.500 mL) of each sample was introduced into a Beckman 119 amino acid analyzer and analyzed according to Spackman et al. (1958). Another aliquot was diluted 1:10 and then analyzed. Some of the samples in 13% Cl_3CCOOH were held for 2–3 months in frozen storage, then evaporated to dryness, and taken up in lithium sample dilution buffer. These samples were analyzed as biological fluid samples on Hamilton HPAN90 resin (Benson, 1971).

RESULTS AND DISCUSSION

The major peak in the chromatogram of the nonhydrolyzed samples was between peaks for aspartic and glutamic acid; its retention time was similar to the retention time of threonine and serine. The peak for the undiluted samples was too large to be measured, thus the samples were diluted 1:10. After hydrolysis, the aspartic acid, glutamic acid, and ammonia peaks for the undiluted samples were too large to be measured. Threonine and serine were measured only in the hydrolyzed samples. Amounts of all of the amino acids increased after hydrolysis (Table I). The increase may have resulted from hydrolysis of small soluble peptides.

Asparagine and glutamine are eluted from the protein hydrolysate column together with threonine and serine (Metzler, 1977). Because the large peak disappeared and aspartic acid and ammonia greatly increased following hydrolysis, we concluded that the large peak was mostly due to asparagine.

We considered the possibility that threonine and serine were bound to carbohydrates in the unhydrolyzed NPN. Unbound serine and threonine could not be measured in the unhydrolyzed samples because their peaks were masked by the large peak believed to be asparagine.

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Table I. Comparison of Amino Acids Measured in the Nonprotein-Nitrogen Fraction before and after Acid Hydrolysis, Jan 5, 1978 (107 Days of Storage) (Means of Duplicate Analyses)

amino acid	g of amino acid/16 g of N recov: not hydrolyzed	hydrolyzed
Asp	10.79	63.20
Thr	0	2.97
Ser	0	4.36
Glu	3.93	6.25
Pro	0.18	0.16
Gly	0.68	1.16
Ala	2.14	2.69
Val	0.78	1.00
Met	0.15	0.45
Ile	0.17	0.22
Leu	0.12	0.19
Tyr	0.46	0.50
Phe	0.97	1.18
Lys	0.08	0.27
His	1.08	1.27
Arg	0.68	0.85
$^1/_2$-Cys	0.09	0.17
Asp	61.48	0
ammonia	0.21	7.43
% total N recov	88.5	74.8

Measurement of serine and threonine in the hydrolyzed samples did not reveal whether or not they were previously bound. When samples of the unhydrolyzed NPN were analyzed on the biological fluids column, asparagine was the major component (Table II). Glutamine was a minor component. The amounts of threonine and serine were close to those found in the hydrolyzed samples, suggesting that there was little binding of these hydroxy amino acids to carbohydrates. Asparagine and glutamine were the only compounds not found in the amino acid hydrolysate.

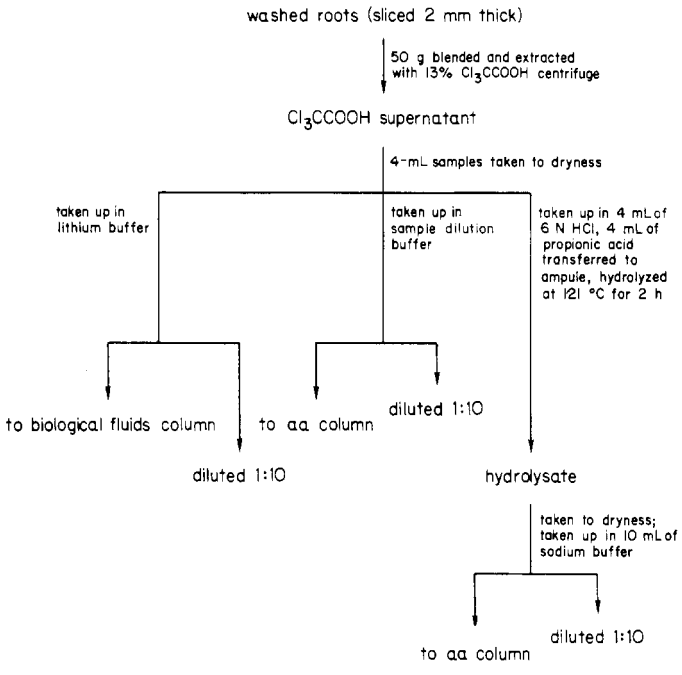

Figure 1. Flow diagram for sampling and analysis.

Table II. Comparison of Analyses from Physiological Fluid Column and Hydrolysate Column

amino acid	g of amino acids/16 g of N recovered: May 18 physiol column	May 18 hydrol column	June 1 physiol column	June 1 hydrol column	June 28 physiol column	June 28 hydrol column
aspartic acid	3.64	7.28	3.21	6.77	3.01	2.92
threonine	3.65	3.70[a]	3.96	5.54[a]	3.85	4.07[a]
serine	1.79	2.29[a]	1.91	2.41[a]	1.87	2.45[a]
asparagine	57.87	50.24	54.75	46.27	50.12	57.10
glutamic acid	5.26	12.00	7.29	11.80	7.13	7.94
glutamine	0.53	0	1.15	0	1.50	0

[a] Determined on hydrolyzed sample because data could not be obtained from unhydrolyzed NPN.

Table III. Regression Equations for Changes in Amounts of Individual Amino Acids Expressed as g of Amino Acids/16 g of N Recovered from the Nonprotein-Nitrogen Fraction of Sweet Potato Roots as a Function of Storage Time[a]

amino acid	regression equation: $A + B$ days $+ (C$ days$)^2$	significance level of regression coefficients: B	C
aspartic acid	$51.18 + 0.1016 - 0.000200^2$	0.0032	0.0286
threonine	$1.54 + 0.0267 - 0.000054^2$	0.0005	0.0161
serine	$1.27 + 0.0615 - 0.000215^2$	0.0005	0.0001
glutamic acid	$9.87 - 0.0476 + 0.000203^2$	<0.0001	<0.0001
proline	$0.69 - 0.0088 + 0.000036^2$	0.0004	<0.0001
glycine	$1.49 - 0.0035 + 0.000005^2$	0.1495	0.5013
alanine	$4.59 - 0.0231 + 0.000005^2$	0.0039	0.0531
valine	$2.50 - 0.0222 + 0.000070^2$	<0.0001	<0.0001
methionine	$0.65 - 0.0085 + 0.000030^2$	<0.0001	<0.0001
isoleucine	$0.88 - 0.0101 + 0.000040^2$	<0.0001	<0.0001
leucine	$0.84 - 0.0087 + 0.000030^2$	<0.0001	<0.0001
tyrosine	$1.20 - 0.0058 + 0.000010^2$	0.0319	0.1392
phenylalanine	$2.08 - 0.0111 + 0.000040^2$	0.0131	0.0101
lysine	$0.41 - 0.0015 + 0.000007^2$	0.1966	0.0360
histidine	$1.85 - 0.0066 + 0.000030^2$	0.0086	0.0002
arginine	$1.68 - 0.0137 + 0.000060^2$	0.0002	<0.0001
cysteine	$0.28 - 0.0017 + 0.000002^2$	0.0771	0.5056
ammonia	$7.67 - 0.0004 - 0.000030^2$	0.9416	0.0735

[a] Twenty-two samples stored from 0 to 281 days.

When NPN of the MeOH extracts was determined, the values averaged about 2% higher than those of the Cl_3CCOOH-extracted NPN, indicating that Cl_3CCOOH was a more effective protein precipitant (data not tabulated). Samples of the MeOH extracts were also analyzed on the biological fluids column. Because the results were essentially the same as those for the Cl_3CCOOH extracts, no further studies were conducted with these samples. The results suggested that essentially no hydrolysis occurred in the Cl_3CCOOH samples.

The amounts of the amino acids were consistently greater after hydrolysis, suggesting that some of the NPN was due to nonprecipitable peptides. There were no seasonal changes in the differences between nonhydrolyzed and hydrolyzed samples, suggesting that the amounts of peptides were constant.

Amounts of the various amino acids changed during storage (Table III). Aspartic acid, threonine, and serine increased during the first part of storage and then decreased. Lysine appeared to increase slightly toward the end of the storage period. Glycine, cysteine, and ammonia did not change significantly during storage. The other amino acids decreased during the first part of storage and increased toward the end.

Recovery of NPN as amino acid N averaged 83.4% for the nonhydrolyzed samples and 83% for the hydrolyzed. Standard deviations were 15.4 and 12.2%, respectively.

These data suggest that the NPN is part of a metabolically active nitrogen pool (Sober, 1970), and that appreciable amounts of nitrogen are stored as asparagine and could be made available for synthesis of amino acids as demanded by the root. The relatively large amounts of aspartic and glutamic acids would be available for transamination reactions. The quadratic change previously reported for the NPN (Purcell et al., 1978a) suggests that this fraction is metabolically active. It may be that the minimum level of NPN represents a stable resting period for the root and the subsequent increase in NPN is caused by the root preparing itself for sprouting. When this nitrogen pool is at its minimum, modifying the storage conditions to maintain that minimum might increase the storage life of sweet potatoes.

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LITERATURE CITED

Adolph, W. H., Liu, H.-C., *Chin. Med. J. (Peking, Engl. Ed.)* **55**, 337 (1939).

Benson, J. V., "New Techniques in Amino Acid Peptide and Protein Analysis", Niederwieser, A., Potaki, G., Eds., Ann Arbor Science Publishers, Ann Arbor, MI, 1971, Chapter 1.

Covington, H. M., Wilson, L. G., Averre, C. W., Baird, J. V., Sorensen, K. A., Proctor, E. A., Beasley, E. O., North Carolina State University Extension Service Publ. AG-09, 1976.

Metzler, D. E., "Bio-chemistry: The Chemical Reactions of Living Cells", Academic Press, New York, 1977.

Mezincescu, M. D., Abo, F. A., *J. Biol. Chem.* **115**, 131 (1936).

Purcell, A. E., Walter, W. M., Jr., Giesbrecht, F. G., *J. Am. Soc. Hort. Sci.* **103**, 190 (1978a).

Purcell, A. E., Walter, W. M., Jr., Giesbrecht, F. G., *J. Agric. Food Chem.* **26**, 6 (1978b).

Sober, H. A., "CRO Handbook of Biochemistry. Selected Data for Molecular Biology", 2nd ed., The Chemical Rubber Company, Cleveland, OH, 1970, pp 1394–1395.

Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* **30**, 1190 (1958).

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Isolation and Characterization of the Major Fraction of Guar Proteins

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The major protein fraction of guar has been isolated in a homogeneous form. It does not contain any phosphorus and has 0.8% carbohydrate. Its sedimentation coefficient is 10.5 S, molecular weight 223 000, and intrinsic viscosity 0.047 dL/g. It consists of at least six nonidentical polypeptide chains. It exhibits an absorption maximum at 280 nm and fluorescence emission maximum at 325 nm and consists mainly of β structure and random coil.

Guar (*Cyamopsis tetragonoloba*) is a commercially important crop. Guar meal, a byproduct of the guar gum industry, contains about 45% proteins. The meal is known to contain some toxic principles (Subramanian and Parpia, 1975; Ambegaokar et al., 1969; Couch et al., 1966), and it has been reported that the protein isolated from it is more toxic to rats than the original meal (Khopkar, 1976; D'Souza, 1972). It was therefore of interest to fractionate guar proteins and study their properties. This paper describes the isolation and characterization of the major globulin fraction of guar proteins.

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MATERIALS AND METHODS

Materials. Guar seeds of the variety FS-277 obtained from the Haryana Agricultural University, Hissar, India, were used. Defatted guar meal (60 mesh BSS) was prepared by the method reported earlier (Nath et al., 1978). The moisture and protein contents of the meal were 8 and 55%, respectively. Sephadex G-200 (40–120 μm) from Pharmacia Fine Chemicals (Sweden), DEAE-cellulose, bovine serum albumin, egg albumin, lysozyme, β-lactoglobulin, and pepsin from Sigma Chemicals (USA), Coomassie brilliant blue and amido black from Schwarz-Mann (Germany), acrylamide from CSIR Centre for Biochemicals

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