Buffalo Gourd Roots: Chemical Composition and Seasonal Changes in Starch Content

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Roots of the perennial xerophytic cucurbit, Buffalo gourd (*Cucurbita foetidissima* HBK.), were monitored for changes in starch content on 17 dates throughout the 1976 season. The starch content of whole root declined slightly at the time of shoot emergence (March 10) and sharply during the period of initial fruit set (May 31). It was highest during mid-season (July 15) and declined again slightly as the vines senesced. A sample collected September 1 provided typical analytical values for crude protein, amino acid distribution, crude fat, sugars, cucurbitacin, and lignin.

Since midcentury, the Buffalo gourd (*Cucurbita foetidissima* HBK.) has been recognized by several authors (Bolley et al., 1950; Curtis, 1946; Shahani et al., 1951) as a potential source of oil and protein for production on arid and semiarid lands. This species, native to southwestern United States and northern Mexico, was described extensively by Bemis et al. (1975). Berry et al. (1976) reported 33.0% crude fat and 32.9% crude protein in whole seeds of this species. Hensarling et al. (1973) determined the nutritive value of Buffalo gourd seed globulins. Weber et al. (1977) evaluated the defatted seed meal as a protein source for weanling mice.

Although the major economic potential of the species lies in its ability to produce oil and protein rich seeds, Berry et al. (1975) reported the presence of root starch in potentially commercial quantity. The plant develops a large perennial tuberous root, presumably used for water storage (Dittmer and Talley, 1964). Three-year-old roots often reach depths of 1 to 2 m, with crown diameters of 20 to 30 cm. By an extractive procedure, 55% (dry weight basis) of the ground whole root was recovered as starch (Berry et al., 1975).

No information is available about variation in starch content of the roots of Buffalo gourd. Starch accumulation during growth has been studied in several tuber and tuberous root crops: cassava (University of Georgia, 1972); yams (Ketiku and Oyenuga, 1973); carrot (Platenius, 1934); and potato (Appleman and Miller, 1926). The conditions controlling carbohydrate storage and utilization in tree crops have been discussed by Priestly (1969).

The purpose of this study was to determine the chemical composition of Buffalo gourd roots and to study the seasonal changes in starch content.

MATERIALS AND METHODS

Plant materials were grown at the University of Arizona Agricultural Experiment Station in Tucson. Roots from plants beginning their second season of growth were harvested periodically from Dec 3, 1975 to Dec 3, 1976. To obtain material for establishment of typical levels of certain root constituents, duplicate roots harvested on Sept 1 were combined. The sample prepared from them was designated as root composition sample (RCS). To determine seasonal fluctuations in starch, crude protein, and lignin, two roots selected randomly were harvested on the 17 dates shown in Figure 1.

Sample Preparation. Roots (and shoots when present) were placed in plastic bags and immediately transported to the laboratory. Each root was washed, towel dried, and weighed. Shoot weights were also obtained. To determine percent dry weight, duplicate portions of each root and shoot were dried overnight at 100 °C. Approximately 100 g of diced fresh root were combined with an equal quantity (v:v) of crushed dry ice and powdered in a food blender. The frozen powder was lyophilized, then ground to a 20-mesh maximum particle size in a Wiley mill, to provide material for subsequent analyses.

RCS Analysis. The analysis was divided into seven major steps, and a schematic diagram of their sequence is shown in Figure 2. In some steps, several constituents were determined.

Step 1. Protein and Amino Acids. The crude protein content of RCS was determined by the micro-Kjeldahl method. Percent protein values were calculated using a conversion factor of 4.00, as determined by the amino acid distribution. The distribution was obtained by ion-exchange chromatography of acid hydrolysates on an automatic amino acid analyzer.

Step 2. Triglyceride Fatty Acids. A portion of RCS was cold-extracted with hexane. The triglyceride fraction was transesterified with acidic methanol. The resulting fatty acid methyl esters were separated by TLC from other hexane-soluble material and analyzed by GLC under the following conditions: 6 ft × 0.125 in. o.d. stainless steel columns packed with 15% DEGS on Chromosorb W; injector ports, 210 °C; flame ionization detector, 200 °C; column oven isothermal, 170 °C; flow rates (N_2), 20 mL/min, (H_2) 30 mL/min, (air) 550 mL/min.

Step 3. Cucurbitacins. Cucurbitacin content was measured by a modification of the technique described by Sharma (1971). Hexane-insoluble residue was suspended in 1:1 (v:v) redistilled chloroform, redistilled methanol, and refluxed for 1 h. After filtration, the filtrate was brought to volume and analyzed spectrophotometrically at 230 nm. The readings obtained were compared to a standard concentration curve prepared with pure cucurbitacin B.

Step 4. Crude Fat. A quantitative determination of the crude fat content of whole root was accomplished by Soxhlet extraction with chloroform. The remaining bulk of RCS was extracted in a similar manner to provide material for analyses in steps 5 through 7.

Step 5. Sugars. A quantitative sugar analysis of defatted powder was obtained by the alkaline ferricyanide colorimetric procedure outlined by Ting (1956). A separate sugar analysis was accomplished by the extraction, purification, and etherification procedures reported by Reineccius et al. (1972). The trimethylsilyl derivatives were separated by GLC under the following conditions: 6 ft × 0.125 in. o.d. copper columns packed with 15% EGS on Chromosorb W; injector ports, 230 °C; flame ionization detector, 180 °C; column oven, 80 to 240 °C; initial hold, 7 min, temperature program, 6 °C/min; flow rates (N_2), 30 mL/min, (H_2) 30 mL/min, (air) 550 mL/min.

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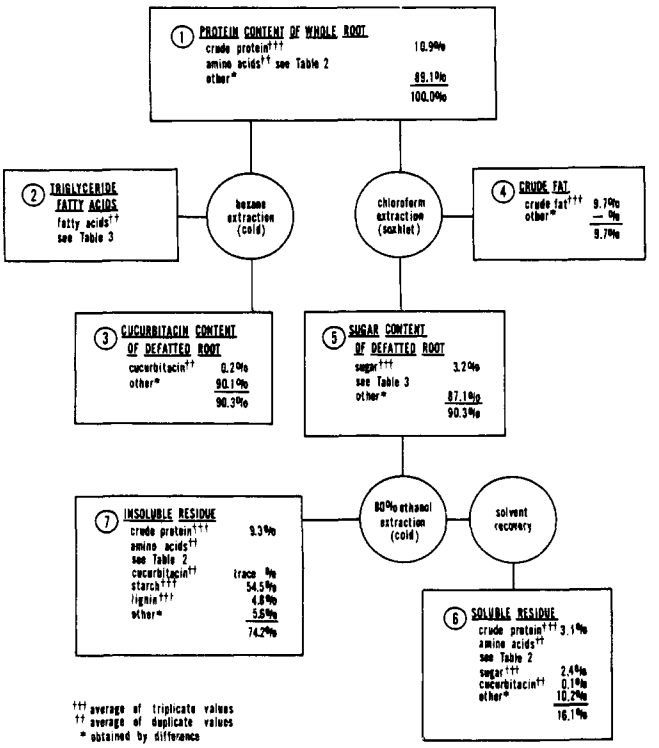

Figure 1. Seasonal changes in starch, protein, and lignin.

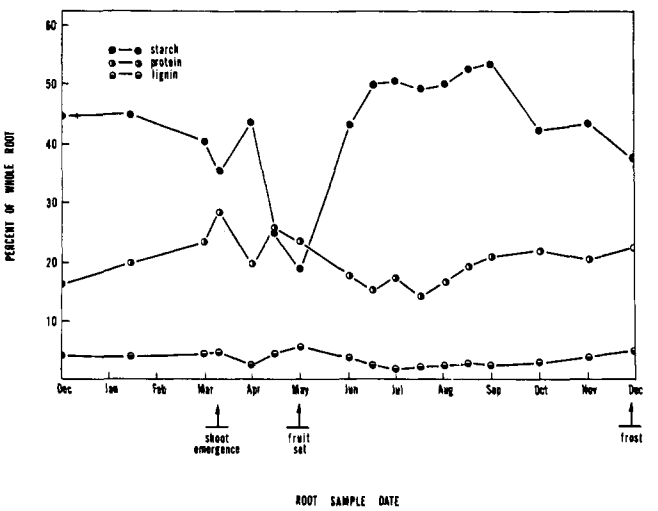

Figure 2. Schematic diagram and results of root composition sample analysis.

Step 6. Constituents of Defatted Material Soluble in 80% Ethanol. Portions of defatted (chloroform extracted) RCS were quantitatively extracted in fritted disc Buchner funnels with 80% ethanol until filtrate aliquots produced a negative Molisch test (AOAC, 1965). The solvent was recovered by rotary evaporation. The resulting residue was subjected to crude protein (conversion factor 3.45), amino acid, quantitative sugar, and cucurbitacin analyses as described above.

Step 7. Constituents of Defatted Material Insoluble in 80% Ethanol. The defatted sugar-free residue was analyzed for its crude protein (conversion factor 4.81), amino acid distribution, and cucurbitacin content. Lignin was determined by the acetyl bromide technique described by Morrison (1972). The polarimetric technique outlined in Whistler (1964) was employed to determine the starch content of the powder.

Analysis of Seasonal Fluctuations. An abbreviation of the RCS analysis procedure was employed to investigate constituent fluctuations in periodic root samples. The freeze-dried root powders (2 per sample date) were subjected to sequential extractions with chloroform and 80% ethanol. The resulting defatted, sugar-free residues were examined for crude protein (conversion factor 4.81), lignin, and starch content as described in step 7 of the RCS analysis.

Table I. Amino Acid Distributions in the Nitrogenous Fractions of Whole Root Powder (Step 1), 80% Ethanol-Soluble Residue (Step 6), and 80% Ethanol-Insoluble Residue (Step 7)

	Step number		
	1	6	7
Lysine[a]	7.7	5.0	9.0
Histidine	2.0	1.7	2.7
NH_3	3.4	4.0	2.7
Arginine	50.5	70.8	31.2
Aspartate	6.4	3.7	8.0
Threonine	1.3	0.1	2.4
Serine	1.9	0.1	3.6
Glutamate	9.9	10.8	8.8
Proline	1.3	0.5	2.8
Glycine	1.8		3.1
Alanine	2.4	0.5	4.4
Cysteine			
Valine	3.8	1.5	7.1
Methionine	0.2	0.5	0.3
Isoleucine	0.1	0.2	0.3
Leucine	2.2	0.4	3.8
Tyrosine	2.8		5.4
Phenylalanine	2.4	0.2	4.3
Tryptophan	*b*	*b*	*b*

[a] Expressed as percentages of amino acid within fractions. [b] Data not available from acid hydrolysates.

Table II. Chromatographic Analysis of Fatty Acids (Step 2) and Sugars (Step 5)

Fatty acids[a]	
Lauric	1.4
Myristic	1.6
Palmitic	21.8
Stearic	3.0
Oleic	3.8
Linoleic	24.8
Linolenic	43.6
	100.0
Sugars[a]	
Glucose	10.0
Fructose	12.8
Sucrose	77.2
	100.0

[a] Expressed as percentages of compounds within fractions.

RESULTS AND DISCUSSION

RCS Analysis. Root composition sample analyses data are presented in Figure 2. All values are reported as percent of whole root on a dry weight basis. Results of the amino acid analyses performed at steps 1, 6, and 7 are listed in Table I. Chromatographic profiles for fatty acid composition (step 2) and free-sugar content (step 5) are summarized in Table II.

Protein. Crude protein analyses and amino acid profiles were obtained at steps 1, 6, and 7. Whole root powder was found to contain 10.9% crude protein with a high concentration of arginine (50.5%). Arginine has also been reported as a major constituent in the protein of Buffalo gourd seed (Weber et al., 1977) and other cucurbit seed proteins (Jacks et al., 1972). Approximately one-third (3.4%) of the nitrogenous material was extracted by 80%

ethanol and proved to be composed mainly of basic amino acids (77.5%), with arginine predominating, (70.8%). The high level of arginine present in both residue portions was unusual. Further study is needed to elucidate the exact nature of this material.

Lipid. Lipid material was extracted from whole root powder at steps 2 and 4. Chromatographic analysis of the hexane extract revealed a triglyceride composition high in unsaturated fatty acids (72.2%). Shahani et al. (1951) reported a Buffalo gourd seed oil profile which was also high in unsaturated fatty acids (89.3%). A crude fat content of 9.7% was obtained by the quantitative Soxhlet extraction of triplicate samples with chloroform.

Cucurbitacins. The presence of cucurbitacins, a class of bitter compounds, was reported in Buffalo gourd roots by Rehm et al. (1957). A measure of cucurbitacin content was obtained in steps 3, 6, and 7. The cucurbitacin content of defatted root powder was found to be 0.2% of the whole root. This value confirms the earlier determination of cucurbitacin content in fresh roots by Rehm et al. (1957). About half (0.1%) of the total cucurbitacin content was soluble in chloroform, while the remainder was extracted by 80% ethanol. A trace of cucurbitacin was found in the 80% ethanol-insoluble residue.

Sugars. The sugar content, determined colorimetrically at step 5, was reported as 3.2% of whole root powder. Chromatographic analysis revealed sucrose to be the predominant sugar (77.2%), with lesser amounts of both glucose and fructose (10.0 and 12.8%, respectively). Three-fourths of the sugar (2.4%) was recovered in the 80% ethanol extract, while the remaining fourth (0.8%) was degraded during the solvent recovery process.

Starch. After sequential extractions with chloroform and 80% ethanol, 74.2% of the whole root powder remained (step 7). Over three-fourths of this sugar-free powder was found to be starch (54.5%). The value determined for starch content concurs with data reported previously (Berry et al., 1975). The whole root powder also contained 4.8% lignin.

Analysis of Seasonal Fluctuations. Throughout the season, the fresh weight of roots ranged from 707 g (April 14) to 4231 g (July 15), while averaging 2169 g per sample date. In general, root fresh weights increased as the season progressed. The fresh weight of shoots, at the time of their emergence (March 10), was 21.5 g and increased to 9020 g (July 1). The average shoot weight per sample date was found to be 3703 g. The percent dry matter of the roots and shoots varied little throughout the season and averaged 26.4 and 19.0% of their fresh weights, respectively.

Seasonal changes in certain root components are displayed graphically in Figure 1. Duplicate values were averaged and reported as percent of whole root on a dry weight basis.

The starch content of the root declined slightly with shoot emergence (March 10; 35.6%) and then sharply at the initial fruit set (May 3; 18.5%). Stored carbohydrates in the root apparently were expended when photosynthetic capability was inadequate to meet the energy requirement of the developing fruit and vines. Priestly (1969) discussed similar fluctuations in the root starch content of apple trees, as influenced by changes in the supply and demand for carbohydrates in other parts of the tree. The starch content of the gourd root was quickly replenished and reached a peak on August 15 (52.0%). As the vines senesced, the starch level of the roots declined. Ketiku and Oyenuga (1973) reported a similar loss of starch in yam tubers at the time of vine senescence.

The crude protein levels of whole root powder averaged 12.8% and ranged from 9.1% (July 15) to 17.9% (March 10). Lignin values ranged from 1.9% (July 15) to 5.3% (May 3) and averaged 3.6%.

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