Webb, R. G.; McCall, A. C. J. Assoc. Off. Anal. Chem. 1976, 55, 746.
Wolff, M. S.; Thornton, J.; Fischbein, A.; Lilis, R.; Selikoff, I. J. Toxicol. Appl. Pharmacol. 1982, 62, 294.
Yakushiji, T.; Watanabe, I.; Kuwabara, K.; Yoshida, S.; Koyama, K.; Kunita, N. Int. Arch. Occup. Environ. Health 1979, 43, 1.
Yamamoto, H.; Yoshimura, H.; Fujita, M.; Yamamoto, T. Chem. Pharm. Bull. 1976, 24, 21681.
Yoshihara, S.; Kawano, K.; Yoshimura, H.; Kuroki, H.; Masuda, Y. Chemosphere 1979, 8, 531.

Received for review February 21, 1984. Revised manuscript received July 30, 1984. Accepted October 9, 1984. This research received financial assistance from the Environmental Protection Agency (CR 806928 and 809764), the National Institutes of Health (ES02798), the Natural Sciences and Engineering Research Council of Canada, the Center for Comparative Medicine, and the Texas Agricultural Experiment Station.

Extraction of a High-Protein Isolate from Jerusalem Artichoke (*Helianthus tuberosus*) Tops and Evaluation of Its Nutrition Potential

Prabhu D. Rawate and Robert M. Hill*

The herbage of Jerusalem artichoke (*Helianthus tuberosus, L*), a plant native to North America, has been demonstrated as a good source for the preparation of a protein isolate that is high in lysine and appears to be a high-quality protein. On the basis of chemical composition, it appears that the resulting residues may provide good ruminant feed.

In a changing world with an increasing population it is essential that the most efficient use of available land be made for the production of food and feed. There is considerable interest in the utilization of renewable carbohydrate resources for the production of alcohol for fuel. The Jerusalem artichoke *Helianthus tuberosus*, with its vigorous growth habit, is receiving attention both as a source of sugar for alcohol production and for possible utilization as a fructose sweetener. This plant, when grown for its tubers, can yield as much carbohydrate as sugar beets or corn grain (Fleming and Groot Wassink, 1979). The forage yield is also very high although a maximum yield of both forage and tubers cannot be obtained simultaneously. The herbage yields, which are approximately equal to the tuber yields, present a disposal or utilization problem (Stauffer et al., 1975). A good deal of promotional claims are available for this native American crop, but only meager information is found in the scientific literature concerning its use as an animal feed. There has been sporadic interest in its cultivation. Early studies were not encouraging and reported yields of only 5–6 tons/acre each for the tubers and the tops (Anderson and Kiesselbach, 1929). In addition, Boswell et al. (1936) reported that harvesting the herbage during the growth period before harvest of the tubers would reduce the tuber yield. Perhaps the greatest deterrent to acceptance as a new crop was that the tubers did not store well and that the principal sugar obtained from the tubers was fructose, which was not in demand as a sweetener at that time. More recently, however, Dorrell and Chubey (1977) have reported yields of tubers as great as 26 tons/ha and one new experimental variety has produced between 38 and 60 tons/ha (Chubey and Dorrell, 1982). In neither case was the herbage yield reported. Preliminary field tests in Nebraska showed that the yield of tubers decreased when forage was harvested at the early bloom stage but chemical analyses of the early harvested forage compared favorably with that of alfalfa (O'Keefe, 1982). Farmers in Minnesota as well as in Nebraska reported no feeding problems with silage made from the herbage. The present study was undertaken when samples of Jerusalem artichoke forage were brought to our service laboratory for forage evaluation. The high protein content of the green forage appeared to be a good source for protein isolate that might have possible uses for diet enrichment.

University of Minnesota Technical College, Waseca, Minnesota 56093 (P.D.R.), and Department of Agricultural Biochemistry, University of Nebraska, Lincoln, Nebraska 68583-0718 (R.M.H.).

MATERIALS AND METHODS

The French white variety of Jerusalem artichoke was grown on marginal soil type without any fertilizer treatment or irrigation at Waseca, MN, during 1983 and the first cutting of the foliage was obtained after six weeks of growth. The second and third cuttings followed at 4-week intervals. The plants were cut about 30 cm from the ground. The entire aerial part (including stems, leaves, and shoots) was composited and chopped before analyses and protein isolation.

Compositional Analyses. Total nitrogen was estimated by the Kjeldahl procedure 46-12 approved by the American Association of Cereal Chemists in 1976 (AACC, 1969). Nitrate nitrogen was determined by the ion-selective electrode method described by Hill and Rawate (1982). Protein was estimated as (total nitrogen – nitrate nitrogen) × 6.25.

Methods described by the Association of Official Analytical Chemists (AOAC, 1980) were used for the determination of ash, crude fiber, ether extract, calcium, magnesium, iron, and zinc and also for solubilization of phosphorus. Phosphorus was determined by the molybdenum blue method (Fiske and Subbarow, 1925). Amino acid analyses were carried out on a 6 N HCl hydrolysate with a Beckman 120C amino acid analyzer as previously described by Hill and Rawate (1982).

Preparation of a Protein Isolate. As in the case of amaranth (Hill and Rawate, 1982), when the total herbage was used as the starting material, pressing alone would not expel sufficient juice for protein isolation. Therefore, it was necessary to prepare an aqueous extract by macerating the chopped herbage with an equal volume of water in a 1 gal size waring blender. A procedure that permits good protein yield in outlined in Figure 1. The protein was

0021-8561/85/1433-0029$01.50/0

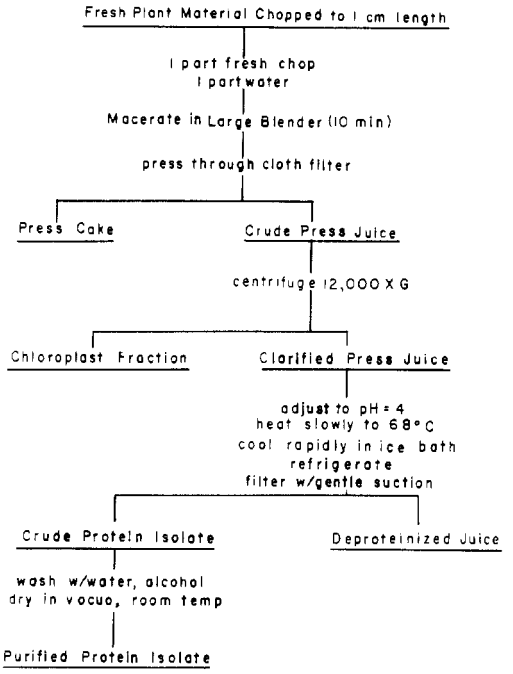

Figure 1. Preparation of protein isolate.

Table I. Feed Constituents in Jerusalem Artichoke Foliage

feed constituent	% composition, dry basis, at harvest dates			
	first cutting, June 24	second cutting, Aug 7	third cutting, Sept 2	uncut until third cutting, Sept 2
crude protein	19.7	20.4	23.6	16.7
ether extract	1.63	1.79	2.16	2.15
ash	17.2	18.5	16.9	14.8
crude fiber	11.2	14.5	15.2	17.8
acid detergent fiber, ADF	33.2	29.5	23.3	41.2
neutral detergent fiber, NDF	34.3	39.0	34.1	40.2
nonstructural carbohydrates	4.7	6.3	6.0	9.6
phosphorus	0.32	0.39	0.34	0.28
calcium	2.00	1.81	2.02	1.68
magnesium	0.53	0.47	0.62	0.40
zinc	0.004	0.005	0.005	0.004
iron	0.014	0.013	0.011	0.015
nitrate as % KNO_3	1.2	0.72	1.5	0.40

precipitated from the clarified juice by adjusting the pH and temperature and was separated by filtration, washed with water and alcohol, and dried in vacuo according to the procedure of Hill and Rawate (1982). It was noted, however, that the protein began to precipitate at a lower temperature than in the case of amaranth. However, the temperature was raised slowly to 68 °C to ensure that maximum precipitation was obtained. After cooling rapidly in an ice bath, the mixture was placed in a refrigerator and the protein was allowed to settle. The bulk of the supernatant could be removed easily with a suction tube and the protein finally separated by filtration with gentle suction. The resulting byproducts are a fibrous residue or press cake, chloroplast material, and waste liquor.

RESULTS AND DISCUSSION

Table I shows the composition of the herbage at different cutting dates. It is to be noted that it contains about 20% protein, which compares well with established forage crops such as alfalfa. The mineral content, particularly calcium, is also satisfactory from a dietary viewpoint. The protein content of the isolates varied from 67 to 76%,

Table II. Feed Constituents in the Fibrous Residue from Jerusalem Artichoke Foliage

feed constituent	% composition, dry basis
crude protein	12.4
ether extract	0.8
ash	16.4
crude fiber	28.3
acid detergent fiber, ADF	49.7
neutral detergent fiber, NDF	46.3
nonstructural carbohydrates	2.3
phosphorus	0.37
calcium	2.62
magnesium	0.48
zinc	0.007
iron	0.03

Table III. Partial Amino Acid Makeup of the Herbage Protein and Herbage Protein Isolate from Jerusalem Artichoke (*H. tuberosus*) Compared with Some Cereal Proteins

amino acid	amino acids, % dry basis				
	Jerusalem artichoke		cereal proteins[a]		
	herbage protein	protein isolate	corn	wheat	soybean
lysine[a]	5.35	5.95	2.3	3.2	6.8
histidine[b]	1.81	1.83	2.5	2.1	2.9
arginine[a]	5.21	6.08	4.8	5.5	7.3
asparatic acid	9.07	8.22	2.2	5.5	15.0
threonine	4.37	4.79	3.7	3.3	3.9
serine	3.95	3.27	1.0	5.0	4.8
glutamic acid	10.46	9.35	30.0	30.0	17.0
proline	4.14	3.87	9.6	12.0	6.1
glycine	5.07	4.79	0	4.4	5.7
alanine	6.33	5.88	3.2	4.3	5.2
methionine	1.39	1.80			
isoleucine[b]	4.60	4.14	6.4	4.0	6.0
leucine[b]	8.33	7.68	15.0	7.0	8.0
tyrosine	2.79	3.70	4.3	3.7	3.0
phenylalanine	5.02	4.58	5.0	5.1	5.3

[a] FAO (1970); Paul and Southgate (1978). [b] Essential amino acids.

which is quite high and compares well with traditional protein concentrates such as fish meal and soybean meal. The fraction of nitrogen recovered from the herbage as an isolate varies from 15 to 27%. However, the unextracted nitrogen is not lost but is retrieved in the chloroplast fraction (average nitrogen about 6%) and in the fibrous residue press cake fraction (average nitrogen 2%).

Any nitrate would be found in the waste liquor. Table II gives data on the composition of the fibrous residue that compares well, nutritionally, with traditional forages with regard to crude protein, crude fiber, TDN, and minerals. Jerusalem artichoke hay or stover are not considered to be very palatable to livestock, but the press cake resulting from the protein isolation may, like silage, be an acceptable feedstuff. Research is needed on this question.

Purified Protein Isolate. Protein isolates were obtained as light colored, odorless, bland-tasting powders that are convenient to handle. Table III gives the partial amino acid content of the protein isolates from the herbage, and the protein isolates are high in the essential amino acid lysine, several times higher than corn and wheat and comparable to soybean meal. The overall amino acid composition also compares well with that of other major cereal proteins.

Table IV gives the yield of dry matter, crude protein, and lysine from Jerusalem artichoke herbage. The data were obtained from small plots for three cuttings during a growing season. The crude protein yield of about 5.4 metric tons/ha compares well with traditional forage crops

Table IV. Potential Yields from Jerusalem Artichoke Herbage Harvested at One-Month Intervals (Yield for Three Cuttings)

constituent	potential yield, kg/ha
dry matter	26 956
protein	5 392
protein in isolate at 15% recovery	808
lysine recovered in isolate	48

such as alfalfa, sorghum, corn, etc. The recovery of protein as an isolate of about 800 kg/ha represents a fraction of the crude protein of the herbage in a highly concentrated form. It appears to store well at room temperature and may be used for a variety of purposes, especially for diet enrichment.

The crude protein content was not enhanced greatly by the nitrate level in the case of early cuttings of Jerusalem artichoke herbage. We have found much higher nitrate levels when immature plants of other species are harvested (corn, sorghum, sudan grass, oats, etc.). In our test plots there was only a slight decrease in tuber yield attributable to harvesting of immature forage. These early cuttings were made well before the tubers began to develop. Such utilization of Jerusalem artichoke herbage may make this crop more attractive to the producer as well as to the processor.

Registry No. Phosphorus, 7723-14-0; calcium, 7440-70-2; magnesium, 7439-95-4; zinc, 7440-66-6; iron, 7439-89-6; nitrate, 14797-55-8.

LITERATURE CITED

AACC. "Approved Methods"; American Association of Cereal Chemists: St. Paul, MN, 1969 (updated Oct 8, 1976).
Anderson, A.; Kiesselbach, T. A. *J. Am. Soc. Agron.* **1929**, *21*, 1001–1006.
AOAC. "Official Methods of Analysis"; Association of Official Analytical Chemists: Washington, DC, 1980.
Boswell, V. R.; Steinbauer, C. E.; Bobb, M. F.; Burlison, W. L.; Alderman, W. H.; Schoth, H. A. *U.S., Dep. Agric., Tech. Bull.* **1936**, *514*.
Chubey, B. B.; Dorrell, D. G. *Can. J. Plant Sci.* **1982**, *62*, 537–539.
Dorrell, D. G.; Chubey, B. B. *Can. J. Plant Sci.* **1977**, *57*, 591–596.
FAO. "Amino-Acid Content of Foods and Biological Data on Proteins"; Food and Agriculture Organization of the United Nations: Rome, 1970; Nutritional Studies No. 24, pp 38–42.
Fiske, C. H.; Subbarow, Y. *J. Biol. Chem.* **1925**, *66*, 375–400.
Fleming, S. E.; GrootWassink, J. W. D. *CRC Crit. Rev. Food Sci. Nutr.* **1979**, *12*, 1–29.
Hill, R. M.; Rawate, P. D. *J. Agric. Food Chem.* **1982**, *30*, 465–469.
O'Keefe, R. B., University of Nebraska, Panhandle Station, Scottsbluff, NE, Personal communication, 1982.
Paul, A. A.; Southgate, D. A. T. "McCance and Widdowson's The Composition of Foods", 4th ed.; Elsevier/North-Holland Biomedical Press: New York, 1978; Section 2.
Stauffer, M. D.; Chubey, B. B.; Dorrell, D. G. *Can. Agric.* **1975**, *Spring*, 34–35.

Received for review June 11, 1984. Accepted October 12, 1984. Partially reported at the 17th American Chemical Society Great Lakes Regional Meeting, St. Paul, MN, June 1–3, 1983. Published as Paper No. 7499, Journal Series, Nebraska Agricultural Experiment Station.

Effects of Temperature on Maillard Reaction Products

Laure M. Benzing-Purdie,* John A. Ripmeester, and Christopher I. Ratcliffe

Equimolecular amounts of D-xylose and glycine were reacted in aqueous solution at temperatures of 22, 68, and 100 °C. Infrared, cross polarization–magic angle spinning ^{13}C NMR of the high molecular weight melanoidins, and ^{13}C solution spectra of the low molecular weight soluble products showed an increase in aromaticity with reaction temperature. The structure of the melanoidin synthesized at 22 °C differed considerably from those synthesized at higher temperature: different types of aliphatic carbons and fewer unsaturated carbons.

Temperature, time, moisture content, concentration, and nature of reactants are important factors in the Maillard reaction (Gomyo et al., 1972; Nursten, 1980–1981; Vernin, 1980). It has been reported that browning increased exponentially with temperature (Shallenberger and Birch, 1975; Vernin, 1980) and that heating melanoidins, the dark brown nitrogenous polymers formed in the reaction, for long periods, causes discoloration and fragmentation (Gomyo et al., 1972). Bobbio et al. (1981) reported the microanalytical data of melanoidins synthesized from glucose and glycine at 70, 80, and 90 °C. Little is known, however, on the effects of temperature on the chemical structure of the Maillard reaction products.

Recently it was shown (Benzing-Purdie and Ripmeester, 1983; Benzing-Purdie et al., 1983), with solid-state ^{13}C and ^{15}N NMR, that remarkable similarities exist between humic substances and melanoidins synthesized from carbohydrates and amino acids at 68 °C. Since the latter temperature is high by soil standards, it was essential to complement the work by further studying the temperature effects on the products of the Maillard reaction. The present communication compares the chemical structures of the high molecular weight melanoidins and the low molecular weight products obtained from the reaction of xylose and glycine at three different temperatures 22, 68, and 100 °C.

Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, Ontario K1A 0C6, Canada (L.M.B.-P.), and Division of Chemistry, National Research Council, Ottawa, Ontario K1A 0R6, Canada (J.A.R. and C.I.R.).

EXPERIMENTAL SECTION

Chemicals. D-Xylose was purchased from Pfanstiehl Laboratories, Inc., Waukegan, IL 60085, and glycine from Sigma Chemical Co., St. Louis, MO 61378.

Synthesis of Melanoidins. *Synthesis of Melanoidin at 22 °C (Am).* A sterile solution of D-xylose (1.5 g) and glycine (0.75 g) in water (10 mL) was kept under sterile conditions at 22 °C over a period of 9 months. A duplicate experiment was run in presence of NaN_2 (0.02%). The melanoidins were recovered after exhaustive dialysis in tubing with a molecular weight cutoff of 12 000, evaporated at 68 °C, and dried over P_2O_5 to give Am: yield, 0.095 g; partial IR 1730 (vw), 1630 (s), 1500 cm^{-1} (vw). Anal.

0021-8561/85/1433-0031$01.50/0 Published 1985 by the American Chemical Society