Evaluation of *Brassica carinata* as a Source of Plant Protein

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Concentrates of plant proteins were prepared from the juice of *Brassica carinata* by heating to 70° and by treating with 2 vol of acetone; yields were 2.3 and 2.6%, respectively. Stage of maturity of the plants affected the yield of protein coagulum. Coagulum yield reached a maximum around 55 days after planting. Amino acid compositions of the two preparations were comparable and similar to published values for proteins of alfalfa, soybean, and other plants. Among the amino acids levels were highest for glutamic acid, aspartic acid, and leucine and lowest for cystine, methionine, tyrosine, phenylalanine, and histidine. Levels of lysine were within the range of those reported for soybeans and other plant proteins. Lysine levels in the protein were not affected by maturity of the plants. The coagulum was a good source of xanthophyll and carotene, but levels of these pigments decreased sharply after their maxima were reached after 55 days of plant growth.

The green leaves of plants are a good source of protein (Kinsella, 1970; Pirie, 1970) and may supply most of the essential amino acids consumed by man. Many proteins of plant and animal origins are derived from amino acids initially synthesized in plant leaves (Akeson and Stahmann, 1968; Stahmann, 1968). However, very few vegetable foods are consumed primarily because of their high levels of protein (Kinsella, 1970). Many more could be successfully exploited as sources of food protein which can be easily extracted from plant tissue (Morrison and Pirie, 1961; Pirie, 1967; Curry and Burns, 1972). The isolation, characterization, stability, yields, uses, and nutritive value of plant protein concentrates have been investigated extensively (Pirie, 1970; Kinsella, 1970; Tao et al., 1972; Witt et al., 1972; Knuckles et al., 1972).

The quantity of plant protein ingested by humans, and possibly by certain monogastric animals, is limited by the large quantities of indigestible fiber and by toxic factors (Kinsella, 1970). The potential and need for the use of plant protein concentrate are recognized but only a relatively few species of plants have been evaluated (Kinsella, 1970; Pirie, 1970). New crops introduced as foods require careful evaluation. *Brassica carinata* was introduced to this country from Ethiopia in 1957 and assigned Plant Introduction Number 243913. This plant, which was grown for the first time in the Southwest during the winter season of 1967–1968, was intended as an oil seed crop, but its lush, tender vegetative growth led to its consideration as a leafy green vegetable crop for fresh, canned, or frozen products (Stephens et al., 1970). These authors reported that the crop's high yield and protein content suggested its potential for protein production. This vegetable has been given the name "TAMU-TexSel" and released as a vegetable crop by Texas Agricultural Experiment Station (Cowley et al., 1972).

Plants of the genus *Brassica* have been tested as a source of plant proteins for supplementation of wheat flour (Garcha et al., 1971). These authors found that when added to wheat flour the protein from sarson (*Brassica campestris*) was superior to that of cowpea (*Vigna sinensis*) and equal to that of palak (*Beta bengalensis*); rat growth was the indicator. Therefore, we have investigated techniques to isolate the protein from the juice of *Brassica carinata* and have determined yield per acre of the crop and yield and chemical composition of the protein.

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EXPERIMENTAL SECTION

The *Brassica carinata* was grown by the Texas Agricultural Experiment Station, Weslaco, Tex. Four plantings over a 2-year period were used in the evaluations. In experiments designed to evaluate the preparation of the juice protein coagulum by heat and by solvent precipitation, plants were harvested just prior to the formation of bloom heads. The protein, amino acids, and other components of the juice coagulum were evaluated. The yields of protein at various stages of plant maturity were measured at 7-day intervals, when the plants had reached a height of 10 to 12 in., about 48 days after planting the seed.

Triplicate random samples, cut by hand 1 to 2 in. above the ground, were immediately trimmed, weighed, and processed. The whole plants were ground with a Model D Fitzmill comminuting machine (the W. J. Fitzpatrick Co., Chicago, Ill.) equipped with a 0.064-in. screen. The juice was pressed by a hydraulic rack and cloth press and the pressure was gradually increased to 4000 psi and held for 10 min.

Samples for whole-plant analyses were taken from each of the cuttings at time of harvest. These samples were hand chopped, dried in a forced-draft oven at 50°, ground in a Wiley mill equipped with a 0.073-in. screen, and redried to constant weight at 70° under vacuum prior to analyses. Samples were taken at the same time for dry matter determination according to the official methods of the AOAC.

In the initial studies, the protein was coagulated by heating the juice or by precipitating it with acetone. One liter (1022 g) of juice was heated to 70° and held overnight at 4°. In the other method, 2 l. of reagent grade acetone was added to 1 l. of nonheated juice with stirring and the mixture was held overnight at 4°. The supernatant was decanted and then the coagulum was filtered through Whatman No. 1 filter paper. The coagulum from both methods was freeze-dried, unwashed after freeze drying, ground with a mortar and pestle, dried in a vacuum oven at 70° to constant weight, and stored until analysis. Analyses performed on the heat-coagulated protein concentrate and not the acetone coagulated material will be indicated in the tables.

Nitrogen was determined by the Kjeldahl method and protein calculated with the factor of 6.25. Amino acids were determined with a Technicon Automatic Amino Acid Analyzer (Technicon Instruments Corp., Tarrytown, N.Y.), on samples hydrolyzed with 6 *N* hydrochloric acid according to Alexander and Block (1960). Results were not corrected for losses of amino acids during acid hydrolysis, but were corrected for recovery of the sample from the ion-exchange column with norleucine as the internal standard.

Table I. Analyses of Protein Concentrate from *Brassica carinata* Prepared by Heating and Acetone Coagulation[a]

Constituent	% Heat	% Acetone
Coagulum[b]	2.3	2.6
Yield protein	53.6	43.2
Ether extract	9.0	5.0
Ash	13.7	22.3
Oxalate	0.54	0.27
Chloride	1.1	1.1
Calcium	0.06	0.05
Phosphorus	0.40	0.77

[a] Values in table represent an average of triplicate determinations on duplicate samples, dry weight basis. [b] Except for coagulum yield, values are expressed as percent of dry coagulum. Coagulum percent yield is based on weight of the juice.

Table II. Amino Acid Composition of *Brassica carinata* Juice Prepared by Heat and Acetone Coagulation[a]

Amino acid	Heat coagulated, mg/100 mg of protein	Acetone coagulated, mg/100 mg of protein
Aspartic acid	11.09	10.18
Glutamic acid	11.53	11.79
Threonine[b]	5.00	4.97
Serine	4.70	4.85
Proline	4.84	5.08
Glycine	5.31	5.61
Alanine	5.78	5.97
Valine[b]	6.28	6.33
Isoleucine[b]	4.97	5.93
Leucine[b]	9.47	9.64
Tyrosine	0.79	0.70
Phenylalanine[b]	2.51	2.47
Lysine[b]	6.04	6.29
Histidine[b]	2.42	2.42
Arginine[b]	5.97	5.85
Methionine[b]	1.44	1.88
Cystine	Trace	0.30
Cysteic acid	1.32	0.54
Ammonia	1.87	1.51
Total	91.33	92.31

[a] Values in the table are based on the recovery from the column of 150 μg of protein in the sample. Values are adjusted for the recovery of norleucine used as an internal standard, but not for the added water of hydrolysis nor for losses of certain amino acids during acid hydrolysis. Tryptophan was not determined due to destruction during hydrolysis. [b] These amino acids are considered to be essential for optimal rat growth (Rose et al., 1948).

For lysine determinations 100-mg samples were enzymatically digested with 5 mg of Pronase (Calbiochem, La Jolla, Calif.) and allowed to react with 2-chloro-3,5-dinitropyridine (Tsai et al., 1972).

Ash and ether extracts were determined according to methods of the AOAC (1970). Calcium and iron were determined on ashed samples by the use of a Perkin-Elmer Model 303 Atomic Absorption Spectrometer (Perkin-Elmer Corp., Norwalk, Conn.). Chloride levels were determined with an Orion Specific Ion Meter (Orion Research Corporation, Cambridge, Mass.,) equipped with a chloride electrode. Xanthophyll and carotene levels in the protein coagulum were determined spectrophotometrically according to the method of Kohler et al. (1967). Oxalates were determined according to the method of Eheart and Hurst (1962).

RESULTS AND DISCUSSION

Results from analyses of crude protein concentrate from *Brassica carinata* plants, harvested about 55 days after planting, appear in Table I. Yields of crude protein coagulum from the juice were 2.3% from heat treated and 2.6% from acetone treated. Protein levels in the crude coagulum were higher in the material prepared by heating, 53.6%, than in that prepared with acetone, 43.2%. Our yields of coagula and levels of protein were lower for both methods of preparation than those for clover and alfalfa reported by Huang et al. (1971). These authors also reported that the precipitation of nitrogenous materials was always greater with organic solvents than with heat. The opposite was true in our studies.

The crude protein concentrate prepared by heating was dark green and that prepared with acetone was light gray to green. The residual liquid was a deep straw color from heat coagulation and deep green from acetone precipitation indicating that most of the pigments in the coagulum had been removed by the acetone. Most of the residual pigment could be removed from the wet coagula by multiple extractions of ethanol followed by hexane. However, removal of the pigments from dried coagula was difficult and we were unable to prepare a white product by extracting the colored, dry coagula.

Lipid or ether extract level of the heat-coagulated concentrate was about double, 9.1%, that of the acetone-prepared coagulum, 5.0%. Apparently extraction of some lipid components by acetone yielded a protein concentrate that was low in lipids. This loss could be important because leaf protein concentrates prepared by heat are high in xanthophyll and carotene which are desirable in feed formulations, especially for poultry.

The coagulum from acetone contained nearly twice as much inorganic material as that from heat; ash contents were 22.3 and 13.7%, respectively. Content of phosphorous was higher in coagulum from acetone but contents of calcium, iron, and chloride were similar in the two preparations. These results, in general, agree with those of Tao et al. (1972).

Oxalate values were 0.54% in the heat-prepared coagulum and 0.27% for the acetone-coagulated material. The values were within the range, 0.27 to 0.87%, reported for the whole plant by Stephens et al. (1970). This range is much lower than that of spinach, 5.42 to 9.81%, and indicates that protein concentrate from the juice of *Brassica carinata* does not contain toxic levels of oxalic acid.

The amino acid compositions were similar for proteins in the concentrates prepared by the two methods (Table II). The results were within the ranges of amino acid composition for plant protein concentrates reported by Tao et al. (1972), Gerloff et al. (1965), and Oelshlegel et al. (1969). In both coagula concentrations were high for aspartic acid, glutamic acid, and leucine and were low for methionine, cystine, phenylalanine, tyrosine, and histidine. Due to the oxidation during hydrolysis, cysteic acid values were interpreted as cystine. Tryptophan, which is completely destroyed during acid hydrolysis, was not determined. Lysine levels were similar to published values for proteins of alfalfa, soybean, and other plants.

The quantity and quality of protein concentrate were dependent upon the stage of maturity and protein level of the plants. Results obtained from experiments to determine the effect of stage of maturity on the quality of the protein concentrate are presented in Table III. For the first sample, taken when the plants were 10–12 in. high, the yield was 7.8 tons of fresh material per acre. Plants were harvested weekly through four harvest periods. Plant yields increased to 19.6 tons per acre but percent juice re-

Table III. Analysis of *Brassica carinata* Plant Protein Concentrate at Different Stages of Maturity Prepared by Heat Coagulation[a]

Days after planting	Plant yield, tons/acre	Moisture in plant, %	Protein in plant, %	Juice yield, %	Coagulum yield, %	Protein in coagulum, %	Ether extract, % of coagulum	Ash, % of coagulum	Lysine, % of protein	Xantho-phylls, mg/100 g of coagulum	Carotene, mg/100 g of coagulum
48	7.8	90.1	27.7	71	1.58	63.5	5.1	9.7	7.6	70.1	43.8
55	13.2	90.6	27.8	67	1.80	69.3	7.7	11.0	7.0	79.2	50.9
62	18.2	89.9	22.6	74	0.53	66.2	1.8	11.7	7.1	24.6	13.0
69	19.6	88.7	20.1	67	0.66	61.9	3.0	11.6	7.4	29.0	15.4

[a] Values in the table represent an average of duplicate determinations on triplicate samples and are expressed on a dry weight basis, except for plant juice and coagulum yields. Plant and juice yields are an average of single observations on triplicate samples, fresh weight basis. Coagulum yield is based on grams of dry coagulum per 100 g of juice.

mained constant. Plant yields per acre were calculated from yields of triplicate plots, 40 in. wide × 10 ft long, at each sampling period. The coagulum and percent protein in the coagulum yield reached a maximum when the plants were about 14–17 in. tall, corresponding to 55 days after planting, and about 7 days prior to the formation of flower shoots or heads. Protein, ether extract, and xanthophyll and carotene in the coagulum were also highest at this sampling. Ether extract, xanthophyll, and carotene decreased as the plant matured. Protein content of the whole plants also decreased as plants matured.

Lysine values in the protein concentrate did not change as the plants matured. For determination of lysine, the α-amino group was complexed with copper and the ϵ-amino group of lysine allowed to react with 2-chloro-3,5-dinitropyridine according to the method of Tsai et al. (1972). Because the reagent does not react with the bound ϵ-amino groups, results indicate available lysine. No accumulation of unavailable lysine was indicated in the protein. This method was a fast, simple technique for estimating lysine in the juice protein concentrate. Results agree reasonably with values for lysine obtained by the amino acid analyzer method and were comparable with lysine values reported by Tao et al. (1972). However, it should be noted that values obtained by the colorimetric method are reportedly higher than those obtained by the amino acid analyzer method (Tsai et al., 1972).

In conclusion, plant protein concentrate was effectively prepared from the juice of *Brassica carinata* by heating to 70° and by treating with 2 vol of acetone. Proteins prepared by both methods were of good quality as indicated by amino acid analysis. Lysine values were within the range of published values for soybean and other plant proteins. Plants harvested just prior to shoot or head formation possessed the highest levels of protein and the quality of the protein was better at this stage of maturity. Plant protein concentrates prepared by heating to 70° were a good source of xanthophyll and carotene.

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