Raffi, J.; Agnel, J. P.; Dauberte, B.; Saint-Lèbe, L. *Starch/Staerke* **1981b**, in press.
Raffi, J.; Dauberte, B.; d'Urbal, M.; Pollin, C.; Saint-Lèbe, L. *Starch/Staerke* **1981c**, in press.
Raffi, J.; Frêjaville, C.; Dauphin, J. F.; Dauberte, B.; d'Urbal, M.; Saint-Lèbe, L. *Starch/Staerke* **1981d**, *33*, 235.
Raffi, J.; Michel, J. P.; Saint-Lèbe, L. *Starch/Staerke* **1980**, *32*, 227.
Raffi, J.; Saint-Lèbe, L.; Berger, G. "Food Preservation by Irradiation"; IAEA: Vienna, 1978; Vol. I, p 517.
Robin, J. P.; Mercier, C.; Charbonnière, R.; Guilbot, A. *Cereal Chem.* **1974**, *51*, 389.
Taub, I.; Angelini, P.; Merritt, C., Jr. *J. Food Sci.* **1976**, *41*, 942.
WHO *W.H.O. Tech. Rep. Ser.* **1977**, *604*.
WHO *W.H.O. Tech. Rep. Ser.* **1981**, *659*.

Received for review January 27, 1981. Revised manuscript received May 26, 1981. Accepted July 8, 1981. Parts of this work were supported by the IFIP, in the field of the CORC program, and by the DGRST (French board for scientific and technical research). Two of us are indebted to the Institut National de la Santé et de la Recherche Médicale (C.J.T.) and to the Centre National de la Recherche Scientifique (C.M.F.) for financial support.

Studies on the Proteins of Poppy Seed (*Papaver somniferum* L.)

H. Srinivas and M. S. Narasinga Rao*

The proximate composition of three varieties of poppy seed, namely, "Dhawla Bada", "Dhawla Chotta", and commercial, showed that the commercial variety had a slightly lower content of protein and oil. Poppy seed meal proteins showed minimum solubility in water around pH 6.6 and a maximum at pH 9.2; in 1 M NaCl and 2% sodium hexametaphosphate solution, maximum solubility was at pH 6.6. Gel filtration on Sepharose 6B gave three peaks, and in polyacrylamide gel electrophoresis one major and several minor bands were observed. In ultracentrifugation four peaks having $s_{20,w}$ values ranging between 0.8 and 1.3, 6.1 and 6.6, 9.1 and 10.3, 14.1 and 15.1 were obtained. The major fraction had an $s_{20,w}$ value of 9.1–10.3. No trypsin inhibitor or hemagglutinin activity was detected in the meal. The proteins of poppy seed were rich in aspartic and glutamic acids and arginine. The amino acid composition did not reveal major differences among the three varieties.

Poppy seeds, from the fruits of *Papaver somniferum* L. (Papaveraceae), are grown in the temperate and subtropical regions of the world. In India, the seeds are mainly grown in the states of Madhya Pradesh, Uttar Pradesh, and Rajasthan. The seed is a good source of protein and oil. Traditionally the seeds are used in food preparations like curries, breads, sweets, and confectionary. Some work is reported on the composition of the oil (Cosovic and Prosteric, 1973; Yarosh and Megorskaya, 1975; Beare-Rogers et al., 1979) and the nutritive value of the proteins (Satyanarayana et al., 1956; Eklund and Agren, 1975). Practically no data are available, however, on the composition and physicochemical characteristics of the proteins of poppy seed. The present investigation deals with the characterization of the proteins from three varieties of poppy seed.

MATERIALS AND METHODS

Materials. Poppy seed varieties "Dhawla Bada" and "Dhawla Chotta" were obtained from the Government Opium Factory, Neemuch, Madhya Pradesh, India, and the commercial variety was obtained through M/s Organon, Calcutta, India.

Preparation of Defatted Poppy Seed Meal. Poppy seed was thoroughly cleaned to remove the impurities and then flaked. The flakes were solvent-extracted 6 times with hexane and air-dried to remove the solvent. The defatted meal was ground to a fine powder and passed through a 85-mesh sieve (BSS).

Central Food Technological Research Institute, Mysore 570013, India.

Proximate Composition. Moisture, protein ($N \times 6.25$), ether extractives, and ash were determined by AOAC (1975) methods. Fiber was estimated by the neutral-detergent fiber method of analysis (Goering and Vansoest, 1970).

Nitrogen Solubility. Two grams of poppy seed meal was mixed with 20 mL of solvent and the pH of the slurry was adjusted to the desired pH by the addition of 2 N HCl or 2 N NaOH. The solvents used were water, 1 M NaCl, and 2% sodium hexametaphosphate (SHMP) in water. The suspension was mechanically shaken for 1 h at room temperature (~28 °C) and centrifuged at 6000 rpm for 30 min, and the pH of the clear supernatant was noted. Aliquots (10 mL) were used for nitrogen determination by the Kjeldahl method. The solubilized nitrogen was expressed as percentage of the total meal nitrogen.

Gel Filtration. Sepharose 6B-100 (Pharmacia Fine Chemicals) which had been equilibrated with 1 M NaCl was packed into a column (1.5 × 100 cm). About 70 mg of protein in 1 M NaCl was applied to the column. The column was eluted with 1 M NaCl solution at a flow rate of 25 mL/h. Fractions (3 mL) were collected in an automatic fraction collector and the absorbance was monitored at 280 nm.

Polyacrylamide Gel Electrophoresis. Electrophoresis was performed according to the method of Davis (1964) using 7.5% gels. In the anionic system, electrophoresis was done in a 0.025 M Tris–glycine buffer of pH 8.3; for the cationic system a 0.05 M β-alanine–acetic acid buffer of pH 4.5 was used. Approximately 200 μg of protein was applied to the gel. Electrophoresis was done for 60 min at 3 mA/tube. The gels were stained with 0.05% Coo-

0021-8561/81/1429-1232$01.25/0

Table I. Proximate Composition of Poppy Seed[a]

constituent, %	"Dhawla Bada"	"Dhawla Chotta"	commercial
moisture	5.0	5.1	5.0
protein ($N \times 6.25$)	22.8	23.5	21.5
ether extractives	49.4	49.3	46.2
ash	5.8	6.0	6.6
fiber	15.6	14.1	15.0
carbohydrate (by difference)	1.4	2.0	5.7

[a] Values are averages of two determinations.

massie blue R-250 overnight and then destained in an isopropyl alcohol–acetic acid–water mixture (5:10:85). Glycoprotein was detected by staining the gels with periodic acid–Schiff's (PAS) reagent and destaining in 1% sodium metabisulfite solution (Clarke, 1964).

Ultracentrifugation. Proteins were extracted in 1 M NaCl solution and extensively dialyzed against the same solvent. Sedimentation velocity runs were done by using 1.5% protein solution in 1 M NaCl at room temperature (~28 °C) at 59 780 rpm in a Spinco Model E analytical ultracentrifuge equipped with RTIC and phase plate Schlieren optics. The $s_{20,w}$ value was calculated by the standard procedure (Schachman, 1959).

Absorption Spectrum. The absorption spectrum of the protein in 1 M NaCl was recorded in a Perkin-Elmer recording spectrophotometer in the range of 240–320 nm.

Protein Concentration. This was routinely determined by measuring the absorbance of the solution at 280 nm. $E^{1\%}_{1cm}$ of the protein was established by plotting absorbance at 280 nm vs. the protein concentration determined by the Kjeldahl method. A factor of 6.25 was used for converting nitrogen content to protein.

Trypsin Inhibitor Activity. About 1 g of poppy seed meal was extracted with 20 mL of 0.1 M phosphate buffer of pH 7.6 for 1 h. After centrifugation, the clear supernatant was diluted 1:10 with the buffer. Trypsin inhibitor activity was assayed in the diluted samples according to the procedure of Kakade et al. (1969).

Hemagglutinin Activity. The hemagglutinin activity of poppy seed meal was tested according to the method of Liener and Hill (1953). The 1 M NaCl extracts of poppy seed meal were dialyzed extensively against 0.9% NaCl. The precipitate formed during dialysis was removed by centrifugation and different aliquots of the supernatant (0.1–0.4 mL) were tested for activity using 0.2 mL of trypsinized rabbit erythrocytes. After overnight storage at 4 °C, agglutination of the red blood cells was estimated by visual observation.

Amino Acid Composition. Poppy seed protein isolate was obtained by extracting the meal in water at pH 8.5, removal of the insolubles by centrifugation and precipitation of the protein in the supernatant at pH 6.0. The protein precipitate was freeze-dried.

The protein content of the isolate was estimated by the micro-Kjeldahl method. The protein was hydrolyzed with 6 N HCl for 24 h at 110 °C. Amino acid analysis was performed by using a Model 118 Beckman amino acid analyzer. Tryptophan was determined according to the method of Spande and Witkop (1967) using *N*-bromosuccinimide.

RESULTS AND DISCUSSION

Proximate Composition. The proximate composition of the three varieties of poppy seed is given in Table I. The analysis was done in duplicate and the results represent the averages. The protein and oil contents varied between 21.5% and 23.5% and 46.2% and 49.4%, respectively. The commercial variety had a slightly lower content of protein and oil. Satyanarayana et al. (1956) have reported 22.3–24.4% protein and 46.5–49.1% ether extractives in different varieties of poppy seed. Eklund and Agren (1975) have reported that a Swedish variety of white poppy seed contained 27% protein and 40% crude oil, while a blue variety had 21% protein and 33% oil.

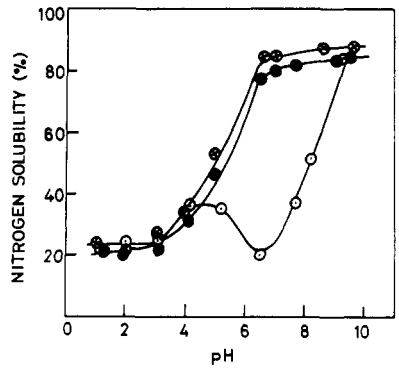

Figure 1. Nitrogen solubility of proteins as a function of pH. (⊙) Water; (⊗) 1 M NaCl solution; (●) 2% sodium hexametaphosphate solution.

Nitrogen Solubility. Nitrogen solubility of the three varieties of poppy seed did not differ significantly. Therefore, an average value could be calculated. Such averaged values obtained at different pHs in water, 1 M NaCl, and 2% sodium hexametaphosphate solution are given in Figure 1. In water, a minimum solubility of ~20% was observed at pH 6.5 and in the range pH 1–3. However, a solubility of ~84% was observed at pH 9.3.

The solubility profile in 1 M NaCl solution was different from that in water. There was only one solubility minimum around pH 2.0. At pH 6.6, about 85% nitrogen could be solubilized. Beyond this pH, no further increase in solubility was observed. Similar solubility profiles have been reported by Eklund and Agren (1975) in water and 1 M NaCl solution.

The solubility profile in 2% SHMP solution was similar to that in 1 M NaCl and different from that in water. At pH 7.5 about 80–83% of the total nitrogen was solubilized. Hanumantha Rao (1977) has reported that cottonseed proteins showed two solubility minima in water and only one minimum in 2% SHMP solution, an observation similar to our findings with poppy seed proteins.

Gel Filtration. The gel filtration pattern of the proteins consisted of three peaks (1, 2, and 3) eluting at 55–58, 105–107, and 141–144 mL (Figure 2). The major protein (peak 2) eluted at 105–107 mL. The proportion of the three fractions varied from 2–13%, 67–76%, and 13–15%, respectively. While there was no difference in the elution volume of the fractions among the three varieties of poppy seed, there was a difference in the proportion. The variety "Dhawla Chotta" had a higher proportion of the major protein fraction (peak 2). Electrophoresis of the individual peaks at pH 8.3 showed that peak 1 stayed as a single band at the top of the gel, peak 2 moved as a single band, and peak 3 split into three bands of high mobility. Only the band from peak 1 stained positive for PAS. These data indicate that poppy seed contains a mixture of high and low molecular weight proteins.

Polyacrylamide Gel Electrophoresis. Electrophoresis at pH 8.3 (Figure 3) showed seven bands; two were of low mobility and the others of high mobility. The latter bands probably were due to low molecular weight proteins. Only the band on top of the gel was positive for PAS stain, indicating its glycoprotein nature. At pH 4.5 the proteins gave a slow-moving major band; the three fast-moving

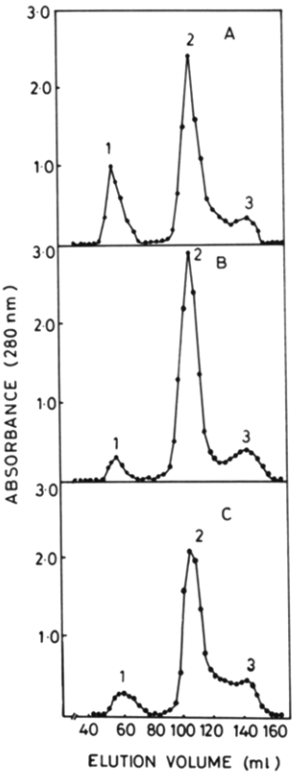

Figure 2. Gel filtration pattern of poppy seed proteins in 1 M NaCl extracts on Sepharose 6B (1.5 × 100 cm). (A) "Dhawla Bada"; (B) "Dhawla Chotta"; (C) commercial.

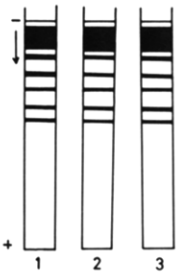

Figure 3. Polyacrylamide gel electrophoresis pattern of poppy seed proteins (0.025 M Tris–glycine buffer, pH 8.3). (1) "Dhawla Bada"; (2) "Dhawla Chotta"; (3) commercial.

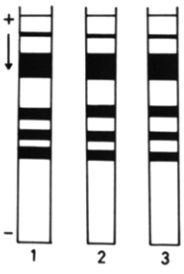

Figure 4. Polyacrylamide gel electrophoresis pattern of poppy seed proteins (0.05 M β-alanine–acetic acid, pH 4.5). (1) "Dhawla Bada"; (2) "Dhawla Chotta"; (3) commercial.

bands were all diffuse (Figure 4). Thus the relative mobility in alkaline and acidic systems showed that poppy seed contained high and low molecular weight proteins, and no differences could be observed either in the mobility or in the number of bands among the varieties.

Ultracentrifugation. Sedimentation velocity pattern of the 1 M NaCl extract of the three varieties consisted in each case of four peaks (Figure 5) having $s_{20,w}$ values ranging between 0.8 and 1.3, 6.1 and 6.6, 9.1 and 10.3, and

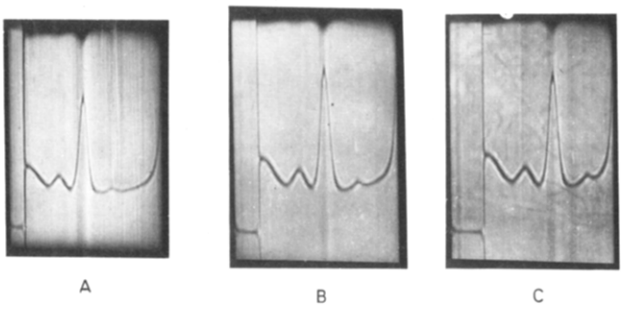

Figure 5. Sedimentation velocity pattern of poppy seed proteins in 1 M NaCl solution. (A) "Dhawla Bada"; (B) "Dhawla Chotta"; (C) commercial.

Table II. Amino Acid Composition of the Poppy Seed Proteins[a]

amino acid	g/100 g of protein		
	"Dhawla Bada"	"Dhawla Chotta"	commercial
aspartic acid	9.27	10.25	9.38
threonine	4.00	3.66	3.44
serine	4.48	4.39	4.40
glutamic acid	24.26	25.40	24.85
proline	4.20	4.37	4.50
glycine	4.19	4.46	4.06
alanine	4.03	4.32	4.46
valine	5.29	5.99	5.38
methionine	2.66	2.54	2.67
isoleucine	4.50	4.35	4.43
leucine	7.22	7.79	7.65
tyrosine	4.23	4.17	3.97
phenylalanine	5.40	4.99	4.80
histidine	2.70	2.54	3.13
lysine	3.51	3.48	3.57
arginine	10.04	10.69	10.21
tryptophan	1.02	1.03	1.00

[a] Values are the averages of duplicate determinations.

14.1 and 15.1. The major protein fraction had $s_{20,w}$ values of 9.1–10.3. The relative proportions of the four fractions were determined from the enlarged tracings of the sedimentation velocity patterns and were 18–20, 10–13, 59–62, and 8–11%, respectively. No significant variations in $s_{20,w}$ values were observed for the different varieties studied.

Absorption Maximum and $E^{1\%}_{1cm}$. The absorption maximum of the proteins of the three varieties was at 278 nm. the $E^{1\%}_{1cm}$ values at 280 nm for "Dhawla Bada", "Dhawla Chotta", and commercial varieties were 8.0, 7.8, and 7.8, respectively. This was estimated from the plot of absorbance vs. protein concentration, which had been determined from the micro-Kjeldahl nitrogen estimation. The aromatic amino acid content of the "Dhawla Bada" variety is slightly higher (Table II).

Trypsin Inhibitor and Hemagglutinin Activity. No trypsin inhibitor and hemagglutinin activity was detected in the three varieties.

Amino Acid Composition. The amino acid compositions of the three varieties of poppy seed proteins are given in Table II. The proteins were rich in glutamic and aspartic acids and arginine. Lysine content was low. There were no major differences in the amino acid composition of the three varieties. The aromatic amino acid contents of the "Dhawla Bada" variety were slightly higher than those of the other two varieties and its valine content was lower.

Eklund and Agren (1975) have reported the essential amino acid composition of two Swedish varieties of poppy seed. There are considerable differences in the values of the two varieties. The values we report here for the Indian

varieties are in good agreement with those for the Swedish varieties, except in the case of lysine, where our values are slightly lower than those reported by Eklund and Agren (1975).

Generally plant proteins are deficient in methionine, having less than 2 g/100 g of protein (Van Etten et al., 1967). However, poppy seeds have a higher content which is comparable with the FAO Reference Pattern for this essential amino acid. Thus poppy seed should be good adjunct to vegetable proteins to enhance their nutritive value.

ACKNOWLEDGMENT

We thank Mr. Sitaram, Centre for Cellular and Molecular Biology, Hyderabad, for help in amino acid analysis.

LITERATURE CITED

AOAC "Official Methods of Analysis", 11th ed.; Association of Official Analytical Chemists: Washington, DC, 1975.
Beare-Rogers, J. L.; Gray, L.; Neva, E. A.; Levine, O. L. *Nutr. Metab.* **1979**, *23*, 335.
Clarke, J. T. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 428.
Cosovic, Cedomiy; Prosteric, Mihoril *Acta Pharm. Jugosl.* **1973**, *23*, 207.
Davis, B. J. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 404.
Eklund, A.; Agren, G. *J. Am. Oil Chem. Soc.* **1975**, *52*, 188.
Goering, H. K.; Vansoest, P. J. *U.S., Dep. Agric., Agric. Handb.* **1970**, *No. 379*.
Hanumantha Rao, K. M.Sc. Thesis, University of Mysore, 1977.
Kakade, M. L.; Simons, N. R.; Liener, I. E. *Cereal Chem.* **1969**, *46*, 518.
Liener, I. E.; Hill, E. J. *J. Nutr.* **1953**, *49*, 609.
Satyanarayana, M. N.; Lakshminarayana Rao, M. V.; Srinivasan, M.; Subrahmanyan, V. *J. Sci. Ind. Res., Sect. C.* **1956**, *15*, 22.
Schachman, H. K. "Ultracentrifugation in Biochemistry"; A.P., Inc.: New York, 1959; p 75.
Spande, T. F.; Witkop, B. *Methods Enzymol.* **1967**, *11*, 498.
Van Etten, C. H.; Kwalek, W. F.; Peters, J. E.; Barclay, A. S. *J. Agric. Food Chem.* **1967**, *15*, 1077.
Yarosh, N. P.; Megorskaya, O. M. *Maslo-Zhir. Promst.* **1975**, *7*, 10.

Received for review April 10, 1981. Accepted August 11, 1981.

Glucosinolates in Crucifer Vegetables: Turnips and Rutabagas

Diana G. Carlson,* Melvin E. Daxenbichler, Cecil H. VanEtten, Harvey L. Tookey, and Paul H. Williams

Twenty-nine cultivars of turnip [*Brassica campestris* L. ssp. *rapifera* (Metzg.) Sinsk.] and twelve of rutabaga [*Brassica napus* L. ssp. *rapifera* (Metzg.) Sinsk.] were analyzed for fourteen glucosinolates. Major glucosinolates in turnip roots are 2-hydroxy-3-butenyl, 2-phenylethyl, and 3-indolylmethyl glucosinolates; in rutabaga roots there are these three plus 4-(methylthio)butyl glucosinolate. Only a few small turnips show a negative correlation between glucosinolate concentration and root weight. Whereas the amounts of individual glucosinolates within a cultivar fluctuated from year to year, there was little change in the relative proportions of these glucosinolates. Both 4-(methylthio)butyl and 5-(methylthio)pentyl glucosinolates are lower in peelings and tops (above-ground parts) of turnips than in peeled roots, whereas 3-butenyl and total glucosinolates were higher. However, 2-phenylethyl and 3-indolylmethyl glucosinolates are concentrated in turnip peelings. Other glucosinolate distributions within the plant are also discussed.

A number of glucosinolates (GS's) that may be regarded as potential toxicants occur in small amounts in cruciferous vegetables. A product from one of these, 2-hydroxy-3-butenyl-GS, is thyrotoxic; others may be thyrotoxic or toxic to the liver or kidney (VanEtten & Tookey, 1979; Nishie & Daxenbichler, 1980; Gould et al., 1980). The toxicity of products from many of the GS's of vegetables is unknown but currently under study. The GS composition of existing cultivars needs to be established to evaluate whether new cultivars might pose health problems because of increased levels of GS's (Senti and Rizek, 1974), as well as to recognize new cultivars of lower GS content. Therefore, the present study of turnips [*Brassica campestris* L. ssp. *rapifera* (Metzg.) Sinsk.] and rutabagas [*Brassica napus* L. ssp. *rapifera* (Metzg.) Sinsk.] was undertaken as a part of a general survey of these crucifers (VanEtten et al., 1980; Daxenbichler et al., 1979, 1980). In addition to those commonly grown in the United States a number of cultivars from Japan and several European cultivars grown primarily as livestock feed were also included.

For a number of years GS's have been known to be present in turnip and rutabaga roots (VanEtten, 1969, and references cited therein). Limited quantitative data has been reported recently: Mullin et al. (1980) analyzed turnip and rutabaga cultivars from Canada and Europe for 5 GS's. We report here quantitative data on 14 GS's in peeled roots of 29 turnip and 12 rutabaga cultivars and indicate the distribution of GS's in tops (above-ground parts), peeled roots, and peelings.

EXPERIMENTAL SECTION

Sample Source and Preparation. Turnip and rutabaga cultivars were grown near Madison, Wi, in 1975, 1978, and 1979 as shown in Tables I and II. Plants were harvested in July or August and then refrigerated until extracted. Refrigeration time ranged from 3 weeks (1975

Northern Regional Research Center, Agricultural Research Service, Science and Education Administration, U.S. Department of Agriculture, Peoria, Illinois 61604 (D.G.C., M.E.D., C.H.V., and H.L.T.), and Department of Plant Pathology, College of Agricultural and Life Sciences and Agricultural Experimental Station, University of Wisconsin, Madison, Wisconsin 53706 (P.H.W.).