Bushuk, W., *Bakers Dig.* **48**, 14 (1974).
Canalco Catalog, "Disc Electrophoresis", Canalco, Rockville, Md., 1973.
Cherry, J. P., *J. Agric. Food Chem.* **22**, 723 (1974).
Cherry, J. P., *Peanut Sci.* **2**, 57 (1975).
Cherry, J. P., Dechary, J. M., Ory, R. L., *J. Agric. Food Chem.* **21**, 652 (1973).
Cherry, J. P., Katterman, F. R. H., Endrizzi, J. E., *Evolution* **24**, 431 (1970).
Cherry, J. P., Mayne, R. Y., Ory, R. L., *Physiol. Plant Pathol.* **4**, 425 (1974).
Cherry, J. P., Ory, R. L., *J. Agric. Food Chem.* **21**, 656 (1973).
Cherry, J. P., Young, C. T., Beuchat, L. R., *Can. J. Bot.* **53**, 2639 (1975).
Dawson, R., *Br. J. Nutr.* **22**, 601 (1968).
Dawson, R., *Anal. Biochem.* **41**, 305 (1971).
Fan, T. Y., Sosulski, F. W., *Can. Instit. Food Sci. Technol.* **7**, 256 (1974).
Frankel, O. H., Bennett, E., "Genetic Resources in Plants—Their Exploration and Conservation", F. A. Davis Co., Philadelphia, Pa., 1970.
Goldblatt, L. A., *J. Am. Oil Chem. Soc.* **48**, 605 (1971).
Gregory, W. C., Gregory, M. P., Krapovickas, A., Smith, B. W., Yarbrough, J. A., in "Peanuts—Culture and Uses", American Peanut Research and Education Association, Inc., Stillwater, Okla., 1973, pp 47–133.
Harris, H., Davis, E. Y., Van de Mark, M. S., Rymal, K. S., Spadaro, J. J., *Agric. Exp. Stn. Auburn Univ. Bull.* **No. 431**, 1 (1972).
Kamra, O. P., *Z. Pflanzenzuecht.* **65**, 293 (1971).
Khan, M. N., Rhee, K. C., Rooney, L. W., Cater, C. M., *J. Food Sci.* **40**, 580 (1975).
McKinney, J. D., Cavanagh, G. C., Bell, J. T., Hoversland, A. S., Nelson, D. M., Pearson, J., Selkirk, R. J., *J. Am. Oil Chem. Soc.* **50**, 79 (1973).
MacRitchie, F., *J. Sci. Food Agric.* **24**, 1325 (1973).
Mattil, K. F., *Food Prod. Develop.* **7**, 40 (1973).
Miller, J., *Science* **182**, 1231 (1973).
Mitchell, J. H., Malphrus, R. K., *Food Technol.* **22**, 95 (1968).
Nelson, O. E., *Adv. Agron.* **21**, 171 (1969).
Neucere, N. J., *Anal. Biochem.* **27**, 15 (1969).
Neucere, N. J., Cherry, J. P., *Peanut Sci.* **2**, 66 (1975).
Pominski, J., Gordon, W. D., McCourtney, E. J., Vix, H. L. E., Gastrock, E. A., *Ind. Eng. Chem.* **44**, 925 (1952).
Pulle, M. W., Ino, K., *J. Milk Food Technol.* **38**, 401 (1975).
Quinn, M. R., Beuchat, L. R., Miller, J., Young, C. T., Worthington, R. E., *J. Food Sci.* **40**, 470 (1975).
Rhee, K. C., Cater, C. M., Mattil, K. F., *J. Food Sci.* **37**, 90 (1972).
Rhee, K. C., Cater, C. M., Mattil, K. F., *J. Food Sci.* **38**, 126 (1973).
Shetty, K. J., Rao, M. S. N., *Anal. Biochem.* **62**, 108 (1974).
Simpson, C. E., Haney, R. L., *Texas Agric. Prog.* **19**, 10 (1973).
Singh, J., Dieckert, J. W., *Prep. Biochem.* **3**, 53 (1973a).
Singh, J., Dieckert, J. W., *Prep. Biochem.* **3**, 73 (1973b).
Smith, A. K., *J. Am. Oil Chem. Soc.* **48**, 38 (1971).
Wolf, W. J., Cowan, J. C., *Crit. Rev. Food Technol.* **2**, 81 (1971).
Ziemba, J. V., *Food Proc.* **8**, 21 (1975).

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Rapeseed Protein Isolates by Countercurrent Extraction and Isoelectric Precipitation

Ahmad S. El Nockrashy,[1] Kumar D. Mukherjee, and Helmut K. Mangold*

A countercurrent procedure for the extraction of protein from defatted rapeseed meal is described. Using 0.02 N NaOH and a meal to solvent ratio of 1:25 as much as 94% of meal nitrogen is extracted. A two-step precipitation, first at pH 6.0 and then at pH 3.6, the two isoelectric points of the protein in the extract, affords two protein fractions, which contain 69.4 and 24.3% of the meal protein, respectively. After washing the curds with water and drying with acetone two highly pure isolates are obtained. Protein isolate I, light grey, contains 92.9% protein and protein isolate II, white, contains 98.6% protein, both on a dry weight basis.

In view of the world-wide protein shortage, oilseeds are becoming of increasing importance as sources of edible proteins (Altschul, 1974). Rapeseed, which is a major oilseed crop of the temperate zones, has so far found little application in the production of protein.

Factors which favor the use of rapeseed protein in human nutrition include: (a) rapeseed is cultivated in countries which do not have suitable agricultural or environmental conditions for growing other oilseeds (Ohlson, 1972); (b) the concentration of essential amino acids in rapeseed protein compares favorably with the amino acid requirements of human adults (FAO, 1957; El Nockrashy et al., 1975a); (c) rapeseed protein is characterized by relatively high contents of lysine (Leslie and Summers, 1975; El Nockrashy et al., 1975b) which is the first limiting amino acid in cereals and other vegetable proteins; and (d) rapeseed protein concentrates having high nutritional value and satisfactory functional properties are well accepted (Ohlson and Tear, 1974).

Problems involved in the use of rapeseed in nutrition include the presence of glucosinolates, which, on enzymatic hydrolysis, yield undesirable and even toxic factors such as isothiocyanates, nitriles, and oxazolidinethione (van Etten, 1969) and the relatively high cellulose content of the seeds (Theander and Åman, 1974). The breeding of new varieties of rape has been only partially successful with regard to reducing the glucosinolate and cellulose contents of the meal (Röbbelen and Leitzke, 1974).

The present investigation was carried out with the aim to develop a process for the preparation of protein isolates free of such undesirable substances. Seeds of two new varieties of rape were used as starting materials, viz., *Brassica napus*, Erglu, a summer variety which is low both in erucic acid and glucosinolates, and *Brassica napus*,

Bundesanstalt für Fettforschung, Institut für Biochemie und Technologie, H. P. Kaufmann-Institut, D-4400 Münster (Westf.), Germany.

[1]Recipient of an Alexander von Humboldt-Fellowship, 1974–1975. Present address: National Research Centre, Dokki, Egypt.

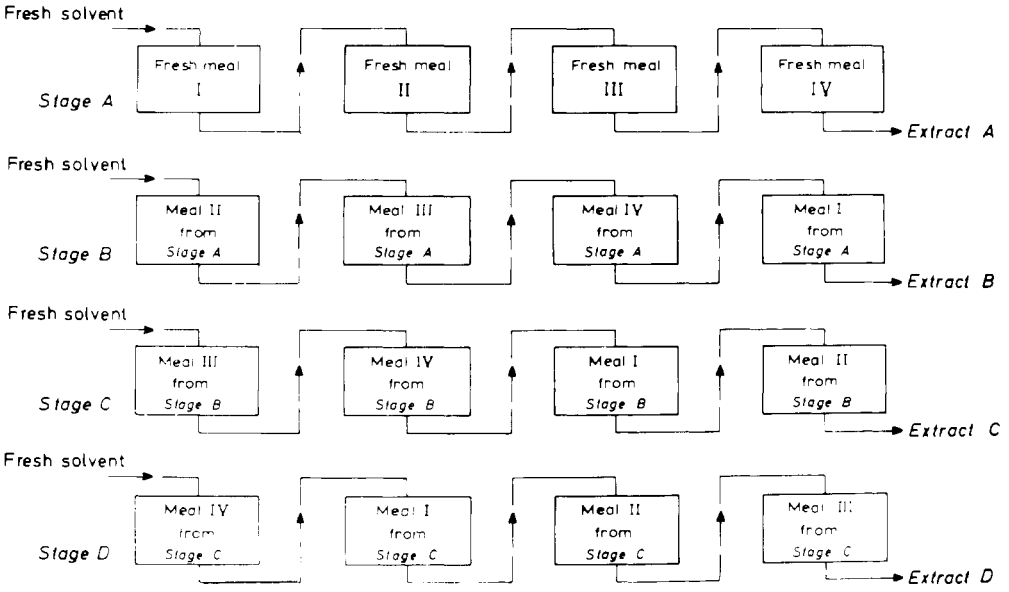

Figure 1. Scheme of countercurrent extraction of defatted meal.

Lesira, a winter variety having a low erucic acid content but a normal level of glucosinolates (Röbbelen and Leitzke, 1974).

We found that countercurrent extraction of defatted rapeseed meal with dilute sodium hydroxide solution results in the dissolution of almost 95% of "meal nitrogen". The proteins in these extracts were found to have two isoelectric points. Stepwise precipitation of proteins at these pH values yields two isolates with a total recovery of about 90% of the meal protein. Washing and drying of these isolates afford products of high purity. The process described appears to be feasible for a large scale production of rapeseed protein isolates.

Nutritional studies with chicks (El Nockrashy et al., 1975a) revealed superior performance of these protein isolates as compared to the corresponding meals, both with regard to growth and protein efficiency ratio. The improved nutritive value of the rapeseed protein isolates is attributed to their favorable amino acid patterns and the absence of glucosinolates.

EXPERIMENTAL SECTION

Materials. Seeds of *Brassica napus*, Erglu (summer 1974), and *Brassica napus*, Lesira (winter 1973/1974), were obtained from local plant breeders. Defatted meals were prepared by extracting the ground seeds with cold hexane, followed by desolventization at room temperature. They were finely ground to pass a 0.2-mm screen, unless otherwise stated. Analyses of the seeds and defatted meals are shown in Table I.

Analytical Procedures. A semimicro-Kjeldahl procedure was used for nitrogen determinations (Clark, 1943); crude protein was calculated as total N × 6.25. Moisture, crude oil, crude fiber, ash, nonprotein nitrogen, and foam stability were determined according to standard procedures (AOAC, 1965; AOCS, 1964). Glucosinolates were determined after their enzymatic hydrolysis to oxazolidinethione and isothiocyanates (Appelqvist and Josefsson, 1967). The color of the protein isolates was determined spectrophotometrically (Fontaine et al., 1945). Molecular weights of the polypeptide chains of the protein isolates were determined by sodium dodecyl sulfate electrophoresis (Weber and Osborn, 1969) with and without the use of mercaptoethanol during incubation. Reference proteins used were human serum albumin, ovalbumin, chymotrypsinogen A, myoglobin from whale, and cytochrome *c*.

Table I. Analysis of New Rapeseed (*B. napus*) Varieties and the Corresponding Meals[a]

	Erglu		Lesira	
	Seed	Meal	Seed	Meal
Crude oil, %	43.7	0.9	44.3	0.8
Total nitrogen, %	3.89	6.61	3.86	6.30
Crude protein, %	24.3	41.3	24.1	39.4
Nonprotein nitrogen, %	0.71	1.20	0.73	1.19
Crude fiber, %	7.4	13.1	10.0	17.9
Ash, %	4.6	8.3	4.1	7.3
Nitrogen-free extract, %	20.0	36.4	17.5	34.6
Oxazolidinethione, mg/g		0.65		13.50
Isothiocyanate, mg/g		0.20		3.20

[a] Calculated on a dry weight basis.

Single Step Extraction of Proteins. Rapeseed meals (*B. napus*, Erglu) having particle sizes of 0.2 and 0.4 mm were extracted with sodium carbonate–sodium bicarbonate buffers, pH 10.0 to 10.6, and sodium hydroxide solutions ranging from 0.02 to 0.05 N. One gram of meal and 10 g of glass beads, 2 mm, were placed in 250-ml Erlenmeyer flasks, and solutions of aqueous alkali were added to give meal to solvent ratios ranging from 1:100 to 1:20. Extraction was carried out at 30 °C by shaking the flasks vigorously for 90 min. The mixtures were then centrifuged at 5000*g* for 15 min. One-milliliter aliquots of the supernatant solutions were taken for the determination of nitrogen. Dissolution of protein was calculated on the assumption that the total volume was fully recovered.

Countercurrent Extraction of Proteins. A schematic representation of the countercurrent extraction procedure followed is shown in Figure 1. Four 5-g samples of meal were weighed into 250-ml Erlenmeyer flasks designated I–IV. In each of the four stages, A–D, the first sample was extracted with 125 ml of 0.02 N NaOH solution (meal/solvent ratio 1:25). Extraction was carried out at 25 °C using an Ultra-Turrax stirrer (Janke und Kunkel KG, Staufen, Germany), for 10 min. Subsequently, the samples were centrifuged at 5000*g* for 15 min. At each stage, the supernatant from one sample was used to extract the next sample, after measuring its volume and taking 0.5-ml aliquots for the determination of nitrogen. At the beginning of each stage, the first sample in the previous stage was moved to the end of the series. The final extracts from the four stages were combined and stored at 5 °C.

Determination of Isoelectric Points. Forty-milliliter aliquots of the protein extracts resulting from the countercurrent extraction were placed in 60-ml centrifuge tubes. These aliquots were titrated with 6 N HCl to various pH values. The curd formed was separated by centrifugation for 30 min at 5000*g*. The supernatant was decanted and its volume and nitrogen content were determined. The overall yield of protein precipitated was calculated as $(V_1N_1 - V_2N_2)/(V_1N_1) \times 100$ [%], where V_1 and V_2 are the volumes of the aliquot before and after precipitation, and N_1 and N_2 are milligrams of nitrogen in 1 ml of V_1 and V_2, respectively. Percentage of protein precipitated was plotted against the pH of the extract in order to determine the isoelectric points. The percentage of protein precipitated was calculated on the basis of true protein content of the meal, which was derived from the difference between the total nitrogen content and the nonprotein nitrogen content of the meal.

Stepwise Precipitation of Proteins. Based on the results obtained in the determination of isoelectric points, 40-ml aliquots of the extracts resulting from the countercurrent extractions were titrated with 6 N HCl to pH 6.0 (Erglu) or pH 5.7 (Lesira) and curds were separated by centrifugation. The pH of each supernatant was then adjusted to pH 3.6.

Alternatively, a 40-ml aliquot of the extract resulting from the countercurrent extraction of Erglu meal was titrated with 6 N HCl to pH 3.6, the curd was separated by centrifugation, and the supernatant was adjusted to pH 6.0.

Analyses of nitrogen in the supernatants were carried out in order to calculate the nitrogen content of the curds. The percentage of meal protein obtained in the curds was calculated from the true protein content of the meals.

RESULTS AND DISCUSSION

In the present study, rapeseed protein isolates were prepared from both defatted Erglu and Lesira meals by a process which involves two major steps. Firstly, rapeseed protein is extracted by a countercurrent procedure using the optimum conditions that were established through single step extractions. Secondly, the proteins are isolated from the extracts by stepwise precipitation at the two isoelectric points. The overall process affords highly pure proteins in excellent yields.

Single Step Extraction of Proteins. It was found that sodium hydroxide solutions give better dissolution of the proteins from rapeseed meals than carbonate–bicarbonate buffers. Highest dissolution (79.7%) is obtained with 0.02 N NaOH at moderate agitation and a meal to solvent ratio of 1:100; it is increased by more than 10% when the particle size is reduced from 0.4 to 0.2 mm. However, when a high-speed homogenizer is used instead of a shaker, dissolution of the protein is hardly affected by the particle size. The use of sodium hydroxide solutions of normalities ranging from 0.02 to 0.2 N has been recommended by several investigators working on the isolation of proteins from rapeseed (Pokorný et al., 1964; Sosulski and Bakal, 1969; Girault, 1973; Kodagoda et al., 1973; Rutkowski and Korolczuk, 1974), cottonseed (El Nockrashy and Frampton, 1967; El Nockrashy, 1973), and soybean (Berardi et al., 1969) by single or multiple step extractions. All of these procedures require rather large volumes of solvent to attain a satisfactory degree of extraction of the proteins.

Countercurrent Extraction of Proteins. In the overall process described in the present study proteins are extracted from defatted rapeseed meal by a countercurrent procedure. This approach is a prerequisite for a continuous large scale production of protein isolates. The major advantage of rather high meal to solvent ratios in a continuous process is that comparatively small volumes of solvent have to be used for a high output. A meal to solvent ratio of 1:25 is chosen because the use of smaller volumes of solvent leads to difficulties in extraction at later steps of stage A owing to high solvent retention by the meal.

Table II. Countercurrent Extraction of Rapeseed (*B. napus*, Erglu) Protein

Extraction stage	Sample no.	Meal nitrogen extracted from each sample, %	Total meal nitrogen extracted at each stage, %
A	I	67.1	
	II	12.0	
	III	31.1	
	IV	26.0	34.1
B	II	90.6	
	III	49.1	
	IV	48.8	
	I	71.0	30.8
C	III	83.4	
	IV	81.9	
	I	82.1	
	II	93.1	20.3
D	IV	97.2	
	I	92.0	
	II	94.2	
	III	91.2	8.6

Sodium hydroxide (0.02 N) which proved to be the most suitable solvent in single step extraction is used in the countercurrent extraction procedure (Figure 1). Table II gives typical results of the countercurrent extraction of proteins from defatted Erglu meal.

Calculations are based on actual recovery of meal nitrogen in the extracts. In stage A, the meal retains about 47% of the solvent. Solvent retention is reduced to 6.2–1.6% in the following stages. Out of the 500 ml of 0.02 N NaOH, a total of 424 ml of extract is recovered. There is no pronounced relationship between the percentage protein extracted from a sample and its position in the entire sequence of extractions. As expected, highest dissolutions are obtained when a sample is extracted with fresh solvent. The total amount of protein dissolved is highest in the final extract of stage A, where 34.1% of the total nitrogen present in the meal is dissolved. The percentage dissolution gradually decreases in each subsequent stage and reaches a level of 8.6% in the extraction at stage D. After the four stages, a total of 93.8% of the meal nitrogen in Erglu meal is extracted. In a similar experiment with defatted Lesira meal, 49.2, 27.2, 14.4, and 1.9% of meal nitrogen are extracted in stages A through D, respectively, the total recovery being 92.7%.

With Erglu meal as an example, Figure 2 demonstrates the relationship between the total amount of nitrogen present in the four samples at the beginning of each stage and the percentage of nitrogen dissolved in the final extract of this stage. It is apparent that the recovery of protein increases with decreasing nitrogen content of the meal samples from stages A to D. The air-dried residues remaining after the countercurrent extraction of Erglu and Lesira meals contained about 5.0% of protein on a dry weight basis.

In another set of experiments with Erglu meal having a particle size of 0.4 mm, the total meal nitrogen dissolved by countercurrent extraction was 91.7%. This shows that

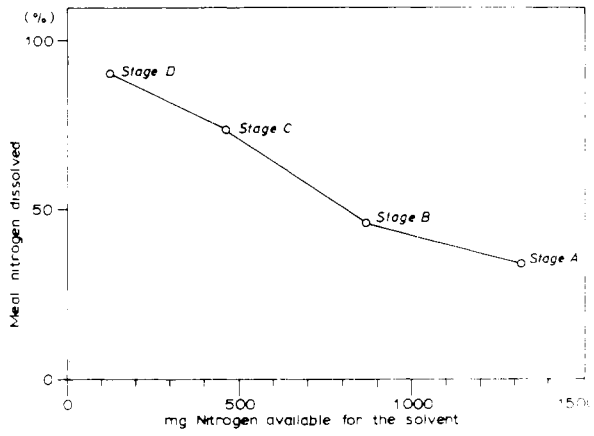

Figure 2. Course of extraction of proteins from defatted rapeseed meals at different stages.

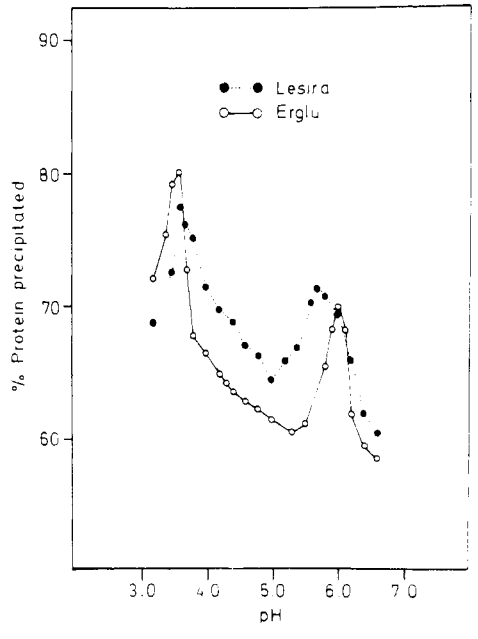

Figure 3. Precipitation curves of rapeseed proteins.

the particle size of the meal is of little significance in this process when high agitation is used.

It is obvious that the countercurrent extraction of protein as described in the present article offers great advantages over single step and multiple step extractions because a high degree of dissolution is attained at a minimum requirement of solvent.

Precipitation of Proteins. In order to establish experimental conditions for the precipitation of rapeseed proteins from the extracts of Erglu and Lesira meals their isoelectric points were determined. Figure 3 shows that the proteins of Erglu and Lesira consist of two major fractions. The proteins of Erglu meal exhibit an isoelectric point at pH 3.6, where 65.7% of the total nitrogen in the extract is precipitated (80.3% of meal protein) and another at pH 6.0, where 57.1% of the total nitrogen in the extract is precipitated (69.8% of meal protein). Similarly, the proteins of Lesira exhibit an isoelectric point at pH 3.6, where 62.7% of the total nitrogen in the extract is precipitated (77.4% of meal protein) and another at pH 5.7, where 57.8% of the total nitrogen in the extract is precipitated (71.4% of meal protein).

There has been some controversy regarding the pH at which the maximum yield of rapeseed protein could be obtained (Pokorný et al., 1964; Sosulski and Bakal, 1969; Girault, 1973; Kodagoda et al., 1973; Rutkowski and Korolczuk, 1974; Finlayson, 1966; Owen, 1973). In view of the present findings it is obvious that a maximum recovery of proteins from rapeseed requires a stepwise precipitation at two isoelectric points, that is at pH 6.0 and 3.6 for Erglu, and pH 5.7 and 3.6 for Lesira. Recent investigations had indicated that rapeseed may indeed contain two main groups of proteins, one of relatively high molecular weight which dissociates into subunits at acid pH values, and another of lower molecular weight (Bhatty et al., 1968; Finlayson et al., 1969; Coding et al., 1970; Janson, 1971; Coding et al., 1972; Girault, 1973). A process for the isolation of proteins based on these findings has, however, not been developed.

Table III. Stepwise Precipitation of Rapeseed Protein

Variety	Precipitation at pH	Meal protein precipitated, %	Total yield, %
B. napus, Erglu	6.0	69.4	
	3.6	24.3	
			93.7
	3.6	79.2	
	6.0	3.6	
			82.8
B. napus, Lesira	5.7	73.2	
	3.6	18.0	
			91.2

Stepwise Precipitation. With the aim to find the optimum conditions for the recovery of proteins from the extracts of Erglu meal, precipitation was carried out at pH 6.0 followed by precipitation at pH 3.6, and vice versa. Table III gives the results of these two experiments.

Precipitation at pH 6.0 results in the formation of a grey curd containing 56.8% of the total nitrogen in the extract (69.4% of meal protein). Further lowering the pH of the supernatant to a pH value of 3.6 affords a light cream colored curd containing 19.9% of the nitrogen in the extract (24.3% of meal protein). Precipitation first at pH 3.6 and then at pH 6.0 results in lower total recovery. The curd obtained at pH 3.6 contains 64.8% of the total nitrogen in the extract (79.2% of meal protein) and that isolated at pH 6.0 yields an additional 3.0% of the nitrogen in the extract (3.6% of meal protein).

Obviously, it is of advantage to carry out the stepwise precipitation first at higher and then at lower isoelectric points. The total recovery of proteins in the two isolates amounts to 93.7% of the proteins in Erglu meal.

Table III also shows results of the stepwise precipitation of proteins from the extract of Lesira meal. Precipitation at pH 5.7 results in the formation of a grey curd containing 59.3% of the total nitrogen in the extract (73.2% of meal protein). Further lowering the pH of the supernatant to a pH value of 3.6 affords a light cream colored curd containing 14.6% of the nitrogen in the extract (18.0% of meal protein). The total recovery of proteins in the isolates amounts to 91.2% of the proteins in Lesira meal.

The major advantage of the present procedure lies in the high recovery of proteins. Other investigators (Sosulski and Bakal, 1969; Girault, 1973; Kodagoda et al., 1973; Rutkowski and Korolczuk, 1974) have reported recoveries of about 50%, only.

Protein Isolates. The purity and the color of the protein isolates can be considerably improved by washing twice with water using a sludge to water ratio of 1:2 and drying with acetone at a sludge to acetone ratio of 1:2. The washed and dried isolates showed only slight pigmentation and their spectra did not exhibit distinct absorption bands.

Table IV. Analysis of Rapeseed Protein Isolates[a]

	B. napus, Erglu			*B. napus*, Lesira		
	Meal	Isolate I	Isolate II	Meal	Isolate I	Isolate II
Protein, %	41.3	92.9	98.6	39.4	99.6	99.3
Lipids, %	0.9	0.00	0.00	0.8	0.00	0.00
Ash, %	8.3	0.78	0.33	7.3	0.04	0.10
Fiber, %	13.1	0.07	0.02	17.9	0.00	0.00
Nitrogen-free extract, %	36.4	6.25	1.05	34.6	0.36	0.60
Oxazolidinethione, mg/g	0.65	0.00	0.00	13.50	0.00	0.00
Isothiocyanate, mg/g	0.20	0.00	0.00	3.20	0.00	0.00
Static foam (Σ)		310	395		411	450

[a] Calculated on a dry weight basis.

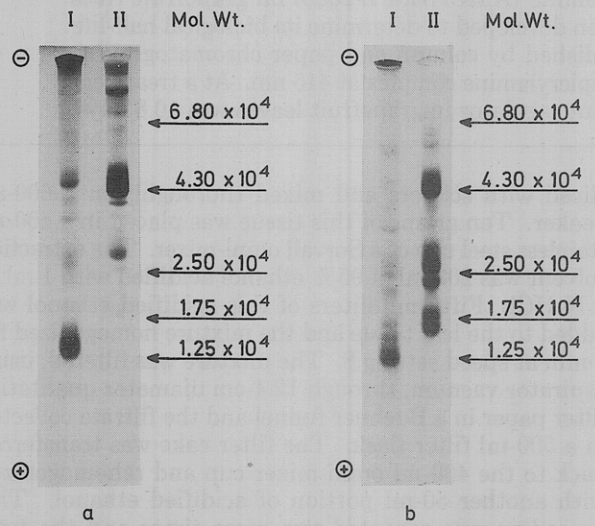

Figure 4. Electrophoretic patterns of protein isolates I and II before (a) and after (b) treatment with mercaptoethanol.

Analyses of protein isolates I and II both from Erglu and Lesira are given in Table IV. The data prove the high purity of these products. The foam stabilities, expressed by the Σ values, are two to three times as high as those reported for soybean protein, and similar to those reported for cottonseed protein (Moshy and Luck, 1964).

The patterns of distribution of various polypeptide chains in the protein isolates I and II from Erglu, as determined by sodium dodecyl sulfate gel electrophoresis, are shown in Figure 4. The electrophoretic patterns of corresponding samples obtained after cleavage of the disulfide bonds with mercaptoethanol are given in the same figure. It is evident that the protein isolates I and II are distinctly different with regard to the composition of polypeptides.

Cleavage of disulfide linkages, both in protein isolates I and II, leads to disappearance of several polypeptide bands, especially of those having higher molecular weights, and formation of low molecular weight polypeptides which are not present in the original samples. Protein isolates I and II from Lesira gave electrophoretic patterns which were identical with those of the corresponding protein isolates from Erglu.

The majority of high molecular weight polypeptides in the protein isolates I and II from both Erglu and Lesira appear to be aggregates of subunits which are covalently bond by disulfide linkages. Further studies are concerned with the structure of rapeseed proteins and the changes they may undergo during isolation according to the process described.

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LITERATURE CITED

Altschul, A. M., Ed., "New Protein Foods", Vol. IA, Academic Press, New York, N.Y., 1974.
Association of Official Agricultural Chemists, "Official Methods of Analysis", 10th ed, Washington, D.C., 1965.
American Oil Chemists' Society, "Official and Tentative Methods", 2nd ed, Chicago, Ill., 1964.
Appelqvist, L.-Å., Josefsson, E., *J. Sci. Food Agric.* **18**, 510 (1967).
Berardi, L. C., Martinez, W. H., Fernandez, C. J., *Food Technol.* **23**, 75 (1969).
Bhatty, R. S., Mckenzie, S. L., Finlayson, A. J., *Can. J. Biochem. Physiol.* **46**, 1191 (1968).
Clark, E. P., "Semimicro Quantitative Organic Analysis", Academic Press, New York, N.Y., 1943.
Coding, L. A., Bhatty, R. S., Finlayson, A. J., *Can. J. Biochem. Physiol.* **48**, 1096 (1970).
Coding, L. A., Downey, R. K., Finlayson, A. J., *Can. J. Plant Sci.* **52**, 63 (1972).
El Nockrashy, A. S., *Grasas Aceites (Seville)* **24**, 361 (1973).
El Nockrashy, A. S., Frampton, V. L., *Biochem. Biophys. Res. Commun.* **28**, 675 (1967).
El Nockrashy, A. S., Kiewitt, M., Mangold, H. K., Mukherjee, K. D., *Nutr. Metab.* **19**, 145 (1975a).
El Nockrashy, A. S., Mukherjee, K. D., Mangold, H. K., *Fette Seifen Anstrichm.* **77**, 451 (1975b).
FAO (U.N.), "Protein Requirements", FAO Nutritional Studies, Vol. 16, 1957.
Finlayson, A. J., *Can. J. Biochem.* **44**, 997 (1966).
Finlayson, A. J., Bhatty, R. S., Christ, C. M., *Can. J. Bot.* **47**, 679 (1969).
Fontaine, T. D., Detwiler, S. B., Jr., Irving, G. W., Jr., *Ind. Eng. Chem.* **37**, 1232 (1945).
Girault, A., *J. Sci. Food Agric.* **24**, 509 (1973).
Janson, J. C., *J. Agric. Food Chem.* **19**, 581 (1971).
Kodagoda, L. P., Yeung, C. Y., Nakai, S., *Can. Inst. Food Sci. Technol. J.* **6**, 135 (1973).
Leslie, A. J., Summers, J. D., *Poultry Sci.* **54**, 532 (1975).
Moshy, R. J., Luck, J. V., Canadian Patent 679 267 (1964).
Ohlson, R., in "Rapeseed. Cultivation, Composition, Processing and Utilization", Appelqvist, L. Å., and Ohlson, R., Ed., Elsevier, Amsterdam, 1972, p 9.
Ohlson, R., Tear, J., Proceedings of the 4th International Rapeseed Conference, Giessen, Germany, 1974, p 663.
Owen, D. F., U.S. Patent 3 758 452 (1973).
Pokorný, J., Sefr, Z., Vodnicka, M., *Prumysl Potrav.* **15**, 285 (1964).
Röbbelen, G., Leitzke, B., Proceedings of the 4th International Rapeseed Conference, Giessen, Germany, 1974, p 63.
Rutkowski, A., Korolczuk, J., Proceedings of the 4th International Rapeseed Conference, Giessen, Germany, 1974, p 637.
Sosulski, F. W., Bakal, A., *Can. Inst. Food Sci. Technol. J.* **2**, 28 (1969).
Theander, O., Åman, P., Proceedings of the 4th International Rapeseed Conference, Giessen, Germany, 1974, p 429.
van Etten, C. H., in "Toxic Constituents of Plant Foodstuffs", Liener, I. E., Ed., Academic Press, New York, N.Y., 1969, p 103.
Weber, K., Osborn, M., *J. Biol. Chem.* **224**, 4406 (1969).

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