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Protein Isolates from Rapeseed:

(Countercurrent Extraction and Isoelectric Precipitation)

by
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Abstract

We have studied to develop a process for the preparation of protein isolates free of isothiocyanate and oxazolidine-thione when defatted meal was extracted with a cold alkaline solution at pHll.O. The rapeseed protein isolates were separated at 0°C using 1% sodium algiante of 500 cps as a precipitation aid, also. The proteins had original colors, namely, a grey curd at pH 6.7, a light cream at pH 5.6 and a yellow cream at pH 5.0, The purity and the color was improved by washing with water and freez-drying with acetone, not at room temperature. A countercurrent procedure was a prerequisite for a continuous large scale production of protein isolates.

Introduction

Rapeseed ranks fifth among the world's oilseeds and is the dominant one grown in Jeju-do. The seed contains about 40% oil and 25% protein.

Defatted meals of rape and similar cruciferous seed crops have a very well amino acid composition and is characterized by relatively high contents of lysine.[1]

However, rapeseed proteins traditionally are not used for food purpose, and it is due to the high content of glucosinolates in rapeseed. Glucosinolate contents up to 7.77% have been reported by Joseffson *et al.*[2]. Glucosinolates are water soluble substances, which, under the influence of myrosinase present in the rapeseed, can be hydrolyzed to give water soluble as well as oil soluble substances such as 1-5- vinylthiooxazolidone and isothiocyanate with a goiter action.[3]

Present investigation was carried out with the aim to develop a process for the preparation of protein isolates free of such undisirable substances. It was found that protein isolates had no production of goitrogenic factors when defatted meal was extracted with a cold alkaline solution to minimize the interaction between glucosinolates and the myrosinase.

As was recently shown by Gillber *et al.*[4], the yield of protein isolates can be considerably increased if a suitable amount of an anionic polymers such as CMC or Sodium hexametaphosphate is added to the extract prior to acidification.

No previous work seems to have been published on the influence of pH on colors of protein isolates or

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on how the yield and properties of rapeseed proteins are influenced by different sodium alginates.

In this paper, we describe the data on the proximate composition of rape and its defatted meal, the dissolution and precipitation behavior of the nitrogen, countercurrent extraction, and isoelectric precipitation as well as amino acid analysis, nitrogen solubility, spectrophotomtric examination of color, and electrophoretic pattern of protein isolates.

Molecular sieve chromatography of different protein fraction.

Materials and Methods

Preparation of rapeseed meal. Rapeseed (*Brassica napus*, winter variety Makino) was obtained from a local breeder, Jeju city in June. About 400g of rapeseed were crushed on a roller mill and the seed hulls removed by wind sifting. The oil from the dehulled seed fraction was extrated with cold hexane (1:20) by shaking the flask and the extracted flakes desolventized without heating. The dehulled, defatted flakes were disintergrated in a hammer mill.

They were finely ground to pass a 0.5 mm screen. Reagents and chemicals. Sephadex G-150, superfine was the product of Pharmacia Fine Chemicals, Sweden. Acrylamide, methylenebisacrylamide, N,N, N', N',-tetramethylenediamine and β-mercaptoethanol were obtained from Wako Chemicals, Ltd., Tokyo, Japan. Ammonium persulfate, analytical grade, was a Fisher product. Bromphenol blue and Coomasie brilliant blue (R-250) were obtained from Mann. Sodium dodecyl sulfate (95%), obtained from E.Merck. Carboxymethylcellulose was the product of Whatman Biochemicals Ltd., London. Sodium alginates of different viscosities were kindly supplied from Sam-Da Co., Jeju. All other chemicals and reagents were of reagent grade and used without further purification.

The standard proteins for molecular weight calibration were: ovalbumin (Calbiochem, La Jolla, CA); chymotrypsinogen Abovine pancreas, cytochrome C-horse heart and myoglobinsperm whale (Schwartz-Mann Biochemical, Orlengeburg, NY); serum albumin-bovine, monomer and dimer, ribonuclease Abovine pancreas (Sigma Chemical Co., St. Louis, MO).

Analysis of chemical composition. A semimicro-Kjeldahl procedure was used for nitrogen determinations[5] and was calculated as total N x 5.5[6]. All protein and nitrogen values were on a dry basis, crude oil, crude fiber, ash, and nonprotein nitrogen were determined according to standard procedures of AOAC.[7] Total phosphorus was determined by the method of Fisk et al.[8].

Calcium was determined by EDTA titration.[9] Glucosinolates were determined after their enzymic hydrolysis to 1-5-vinylthiooxazolidene and isothiocyanate.[10]

Nitrogen solubility of meal proteins. A weighted amount of rapeseed meal was dispersed in an amount of deionized water, calculated to give a final meal to liquid ratio of 1:20. The pH of the slurry was adjusted to the predominated value by the addition of aqueous sodium hydroxide or hydrochloric acid. The extraction was allowed to proceed with stirring for 30 min using a magnetic stirrer (Yanaco Model 8, Japan) at room temperature. Insoluble materials were removed by centrifugation at 5000 x G for 15 min at 0°C in an automatic high speed refrigerated centrifuge (Hitachi 18 PR-3, Japan). The supernatant was lyophilized.

Precipitation of alkaline extracts. Hydrochloric acid was added, with through stirring to the meal extract until the predetermined pH of the mixture was reached. In some experiments CMC or sodium alginates was added before the addition of hydrochloric acid. The precipitate was recovered by centrifugation at 5000×G for 15 min at 0°C. The nitrogen yield of isolates was calculated as: (total amount of nitrogen in the precipitate)/(total amount of nitrogen in the quantity of extract used to prepare the precipitate).

Countercurrent extraction of meal proteins. A schematic representation of the countercurrent extraction procedure followed is shown in Figure 1. Four 5g samples of meal were weighed into 250-ml Erlenmeyer flasks designated 1-4. In each of the four stages, 1-4, the first sample was extracted with 100 ml of 0.02 N NaOH solution (meal/solvent ratio 1:20). Extraction was carried out at 4°C in a magnetic stirrer for 10 min. Subsequently, the slurry was

Solvent Stage Ⅰ	→ meal 1.	→ meal 2.	→ meal 3.	→ meal 4.	— Extract Ⅰ
Solvent Stage Ⅱ	→ meal 2. from Stage Ⅰ	→ meal 3. from Stage Ⅰ	→ meal 4. from Stage Ⅰ	→ meal 1. from Stage Ⅰ	— Extract Ⅱ
Solvent Stage Ⅲ	→ meal 3. from Stage Ⅱ	→ meal 4. from Stage Ⅱ	→ meal 1. from Stage Ⅱ	→ meal 2. from Stage Ⅱ	— Extract Ⅲ
Solvent Stage Ⅳ	→ meal 4. from Stage Ⅲ	→ meal 1. from Stage Ⅲ	→ meal 2. from Stae Ⅲ	→ meal 3. from	— Extract Ⅳ

Fig. 1. Scheme of countercurrent extraction of defatted meal.

centrifuged at 5000×G for 15 min at 0°C. 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, The final supernatants were lyophilized.

Determination of isoelectric points. The determination of isoelectric points were carried out by the method of El Nockrashy *et al.*[11] An alkaline extract (20 ml) of defatted meals was pipetted into each of 50-ml centrifuge tubes, and 6 N HCl was added dropwise to each tube to various pH values. The mixture in each tube was stirred magnetically for 5 min at 4°C and centrifuged at 5000×G for 15 min at 0°C. The supernatant was decanted and its volume and nitrogen were determined. The overall yield of protein precipitated was calculated as $(V^1N^1V^2N^2)/(V^1N^1)\times 100$ (%), where V^1 and V^2 are the volumes of the aliquots 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.

Stepwise preparation of protein isolates. Based on the results obtained in the determination of isoelectric points, 35-ml aliquots of the extracts resulting from the countercurrent extractions were titrated with 6 N HCl until pH value was 6.7 at 4°C and curds were separated by centrifugation (5000×G) for 15 min at 0°C (A). The second protein isolate (B) was collected by adjusting the pH of the supernatant to 5.6. After centrifugation, the third protein isolate (C) was obtained when the pH of the supernatant decanted was 5. 0. Isolates, A′, B′ and C′, were prepared from the alkaline meal extract as shown schematically in Figure 2. Isolate A′ was prepared by adding 12 ml of a 1% aqueous solution of CMC to 100 ml of supernatant A. Isolate B was prepared by adding 6 ml of a fresh 1% solution of sodium alginate of 500 cps to the supernatant B. Isolate C was prepared by adding 4ml of a fresh 1% sodium alginate of 200 cps to 100 ml of the supernatant C. The CMC and sodium alginates used were chosen to give a high nitrogen recovery. All of the protein isolates were washed with water (except a part) using

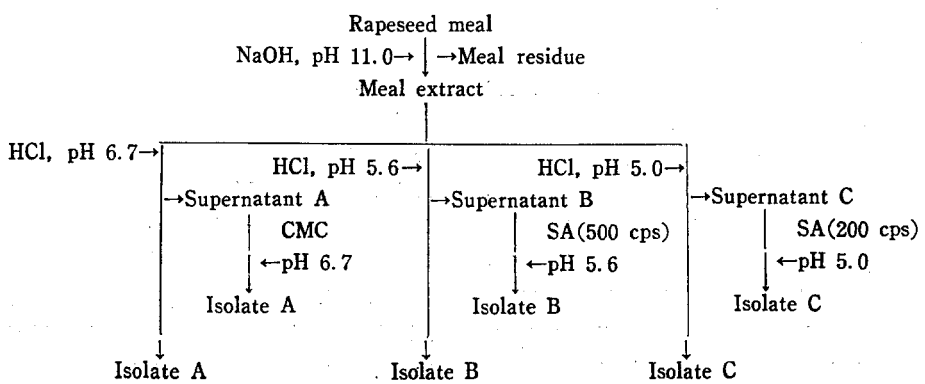

Fig. 2. Scheme for the preparation of protein isolates.

a sludge to warer ratio of 1:2 and then lyophilized with acetone at a sludge ratio of 1:2.

Spectrophotometric examination of isolates A, B and C. The colors of the isolates were determined by the method of Fontain *et al.*[12] Solutions of various proteins were prepared by dissolving 100 mg in 25 ml of 0.02 N NaOH. Occasionally it was necessary to centrifuge to obtain clear solutions.

Nitrogen solubility of protein isolates. Nitrogen solubility of isolates, A, B and C, was determined by stirring 100 mg of solid with 10 ml of water: sodium hydroxide or hydrochrolic acid solution was added dropwise to give a range of pH value from 2.0 to 10.0. The slurry was stirred magnetically for 5 min and centrifuged at 5000 x G for 15 min to separate solid and supernatant satisfactorily, The supernatant was analyzed for nitrogen and the percentage of soluble nitrogen was calculated at each pH value.

Amino acid analysis. The isolates, A, B and C, were hydrolyzed in 6 N HCI for 24 hr at 110°C with amples, evapolated to dryness and dissolved in pH 2.2 citrate buffer. The hydrolyzates were analyzed in a Hitachi amino acid analyzer, Model KLA-5A. Cysteine was estimated as cysteic acid after performic acid oxidation by the method of Schram *et al.*[13]. Tryptophan was determined according to the method of Inglis *et al.*[14].

Sodium dodecyl sulfate-gel electrophoresis. Electrophoresis was carried out on 10% acrylyamide gels in the presence of 1% SDS according to the procedure of Weber *et al.*[15]. The proteins were incubated at 37°C for 2 hr in 0.01M sodium phosphate buffer, pH 7.2. Electrophoresis was performed at a constant current of 8 mA per gel with the positive chamber electrode in the lower chamber.

Isoelectric focusing. Isoelectric focusing was carried out according to the method of Wrigley[16] over the pH range of 3 to 10 using ampholine (LKB Instrument Inc.) in 6M urea. The electrophoresis was done at 220 volts with an initial current of 60 mA that dropped to 10 mA in 30 min and continued for a further 60 min. The gels were then stained for 1 hr with 0.2% bromphenol blue in ethanol:acetic acid (50:45:5 by volume) and destained with ethanol:acetic acid (30:65:5 by volume) as reported by Awdeh.[17]

Moleular sieve chromatography. The protein extract, the isolates and the supernatants were analyzed by molecular sive chromatography on Sephadex G-150 equilibrated in 0.01M ammonium acetate/ammonia buffer of pH 11.0 containg 0.1M NaCl. The column (2.5 x 40.0cm) was calibrated with the standard proteins. The absorbance at 280 nm was used for protein assay and the orcinol H_2SO_4 method, giving a colored complex adsorbing at 540 nm and described by Svennerholm[18], for hexose assay. The sample size was 2.5 ml, the flow rate 14 ml/hr and the fraction volume 2.5ml.

The lyophilized isolates were prepared for analysis by dispersing 30 mg in 2.5ml of the buffer (pH 11.0). Undissolved material was removed by centrifugation and the supernatant was dialyzed against the buffer. The dialyzed solution was used as sample.

Results and Discussion

Chemical composition of the meal. As shown in Table 1 the proximate analysis of the cold hexane-defatted rapeseed indicated the meal contained about 40% of protein and showed the least toxic factors and, if processed to a food grade, would have many direct uses as protein concentrates. The fiber levels between rapeseed and its meal were evident that the

Table 1. Proximate composition of rapeseed and the corresponding meal

	B. napus, winter variety, Makino	
	Seed	Meal
Crude oil,%	44.6	3.0
Total nitrogen,%	4.49	7.27
Crude protein,%*	24.7	40
Nonprotein nitrogen,%	0.6	1.23
Crude fiber,%	11.0	4.32
Ash,%	5.8	8.2
Nitrogen-free extract,%	13.9	44.48
1-5-vinylthiooxazolidone, mg/g	14.2	0.46
Isothiocyanate, mg/g	5.2	0.22

* Protein (N x 5.5) on a dry weight and oil-free basis.

development of techniques for removal of seed hulls before processing would enhance the utilization of the meal for non-ruminant nutrition.

Solubility profile and precipitation. The influence of pH on the relative solubility of the nitrogen in rapeseed meal is shown in Figure 3. The nitrogen solubility curves have a rather complicated protein composition and contain with isoelectric points in the pH range 4-11. It shouldbe realized that the curves produced in Figure 3, do not represent equilibrium data. From these results it is evident that rapeseed behaves quite differently from more well-known protein-rich oil seeds like soybean and sunflower (cf. e.g., Fontain *et al.*[19]). The meal showed relatively high nitrogen solubility at pH 11.0, 73.2%. The use of sodium hydroxide solution of normalities ranging from 0.02 to 0.2 N has been recommended by several investigators working on the isolation of proteins from rapeseed by single or multiple step extractions. All of these procedures require rather large volume of solvent to attain a satisfactory degree of extraction of the proteins.

With reference to the solubility behavior of the proteins (cf. Fig. 3) a technical process for the preparation of a rapeseed protein isolate most likely has to be based on a protein step conducted at a fairly high pH. Results from precipitation experimnts with extract solutions prepared at pH 11.0 are given in Figure 4.

As the results show, the precipitation maximum in this case was very broad, covering a much wider

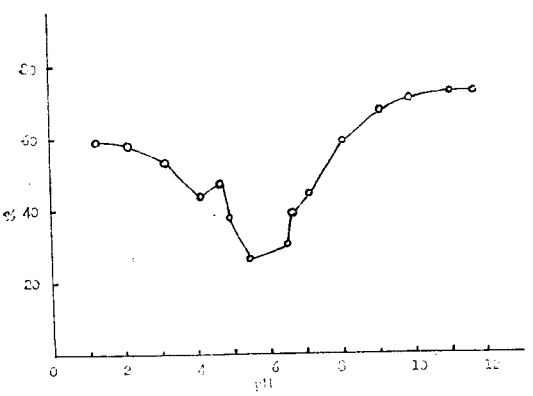

Fig. 3. Dissolution of nitrogen as a function of pH during extraction of rapeseed meal.

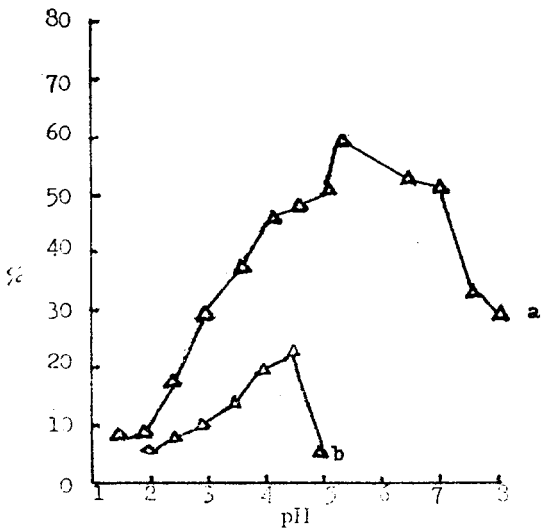

Fig. 4. Influence of pH on the precipitation of a protein extract solution, prepared at pH 11.0 from rapeseed meal:

(a) precipitation in one step; (b) precipitation of the supernatant obtained by first carrying out a precipitation step at pH 5.6.

pH range than the solubility curve (Fig. 3). This, most probably, is partly a consequence of the fact that the extract contained a large number of different proteins having widely different molecular weights and isoelectric points.

The maximum yield was rather low, and more than 40% of the dissolved proteins remained in the superatant. Attempts to study the feasibility of increasing the yield by carrying out the precipitation in two successive steps were made. In the first step, hydrochrolic acid was added to lower the pH to 5.6. The supernatant obtained, after removing of the precipitate was then used to study the influence of the pH of precipitation on the recovery of nitrogen in the second step. As can be seen precipitation occurred in a rather limited pH range only. The maximum recovery in the second step amounted to 22% of the dissolved nitrogen. This means that the theoretical maximum yield of nitrogen in this two step precipitation process was 73%. The results obtained are in agreement with the high solubility of rapeseed protein (cf. Fig. 1) and with previous literature data. Thus, Girauet[20] has reported a maximum recovery of 56% of the dissolved proteins. Pokorny et al.[21] 49% and Gillberg

et al.[22] 55%.

Sodium algintate as a precipitation aid. As was recently shown by Gillberg *et al.*[23], the yield can be considerably increased if a suitable CMC or HMP (sodimhexametaphosphate) is added. Results showing the influence of the pH of precipitaion on the nitrogen yield at various additions of sodium alginate are given in Figure 5. As can be seen, the larger the amount of SA added, the narrower were the precipitation maxima. It can also be seen thas the precipitation maxima were displaced towards lower pH values when the addition of SA was increased. This displacement is in agreement with the assumption that the effect of SA depends on its formation of neutral complexes with the proteins and that the complex formation involves the reaction between carboxyl groups on SA and opsitively charged amino acid residues on the proteins. The high nitrogen yields obtained at the largest additions of SA indicate a co-precipitaon of a large proportion of the nonprotein nitrogen containing substances in the extract.

The results in Figure 6 are from experiments with three samples of SA, differing in viscosity. These experiments were carried out to compare the effecti-

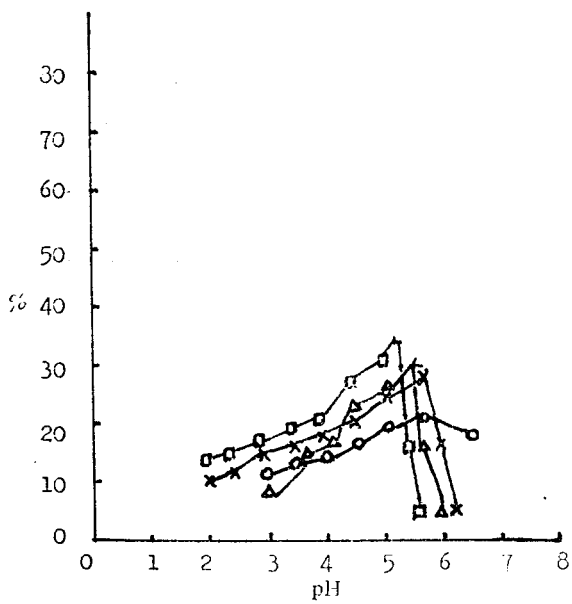

Fig. 5. Influence of pH on the precipitation of a meal extract to which was added different amount of SA (1000 cps): 0, 0.01 SA/protein; x, 0.05; △, 0.1; □, 0.3.

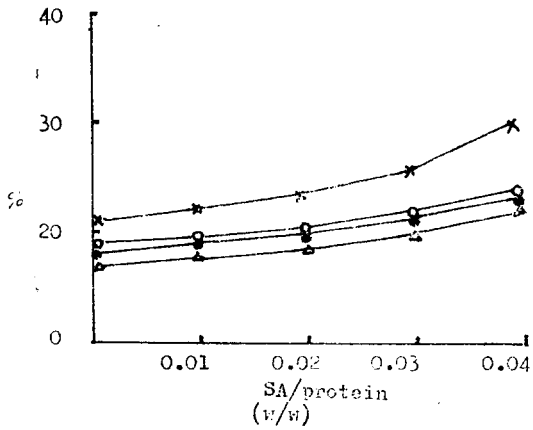

Fig. 6. Influence of the addition of SA of different viscosity to a meal extract on the precipitation at pH 5.0 of the mixture. (0, 1000 cps;x, 500 cps; △, 200 cps); (0, CMC)

vity of the different SA samples and were not extended to additions of SA, where its protective colloid action was noticeable. The results imply, although small differences were obtained, that SA with a highest viscosity (1000 cps) are net as effective as those with a low viscosity.

Very low product losses were ebtained when isolates prepared with SA were subjected to washings with deionized water. This is of great practical importance, because in an isolate process the product must be washed several times to be freed from contaminants, e.g., glucosinolates, present in the mother liquor.

Countercurrent extraction of meal proteins. In the overall process in the present study proteins are extracted defatted rapeseed meal by a countercurrent procedure. This approach is a prerequisite for a continuous lage production of protein isolates and concentrates.

The major adventage 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.

Table 2 gives typical results of the countercurrent extraction of proteins from the meal.

Calculations are based on actual recovery of meal nitrogen in the extracts. In stage 1, the meal retains about 32% of the solvent. Solvent retention is reduced to 7.8-0.4% in the following stages.

Table 2. Countercurrent extraction of rapeseed protein

Extraction stage	Sample No.	Meal nitrogen extracted from sample	Total meal nitrogen extracted at eachstage, %
I	1	71,9	
	2	17.5	
	3	32.4	
	4	28.2	37.5
II	2	91.4	
	3	51.6	
	4	46.5	
	1	74.8	32.7
III	3	81.1	
	4	87.3	
	1	83.6	
	2	94.5	18.8
IV	4	95.8	
	1	94.0	
	2	91.8	
	3	93.2	7.2

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 1, 37.5% of the total nitrogen present in the meal is dissolved. After the four stages, a total of 96.2% of the meal nitrogen is extracted.

The air dried residues contained about 3.7% of pretein on dry weight basis. Calcium in the residues was over 0.25%, and total phosphorous showed 0.83

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

Stepwise precipitation of meal extract. In order to establish experimental conditions for the precipitation of rapeseed proteins from the extract of the meal their isoelectric points were determined. As shown in Fig. 4 their isoelectric points were 6.7, 5.6 and 5.0. There has been some controversy regarding the pH at which the maximum yield of rapeseed protein could be obtained.

In view of the present findings it is obvious that a maximum recovery of proteins from rapeseed requires a stepwise precipitation at three isoelectric points. Recent investigations had indicated that rapeseed may indeed contain three main groups of proteins, one of relatively high molecular weight which dissociates into subunits at acid pH values, and another of lower molecular weight of protein. Precipitation at pH 6.7 results in the formation of a grey curd. Further lowering the supernatant to a pH value of 5.6 affords a light cream colored curd. At pH 5.0 a yellow cream colored curd was formed. The purity and the color of the protein isolates can be considerably improved by washing twice water using a sludge to water ratio of 1 : 2 and drying acetone.

Analyses of protein isolates A, B and C are given in Table 3. The data prove the high purity of products.

Spectrophotometric examination of color. Figure 7 shows representative transmittance curves (360 to 680nm). They are essentially smooth. The curves

Table 3. Chemical composition of protein isolates

	Brassica napus, Makino (winter variety)		
	Isolate A	Isolate B	Isolate C
Protein,%*	99.7	99.8	99.5
Lipid,%	0.0	0.0	0.0
Ash,%	0.2	0.1	0.2
Fiber,%	0.0	0.0	0.0
Nitrogen-free extract,%	0.44	0.26	0.37
1-5-vinylthiooxazolidone, mg/g	0.0	0.0	0.0
Isothiocyanate, mg/g	0.0	0.0	0.0

*

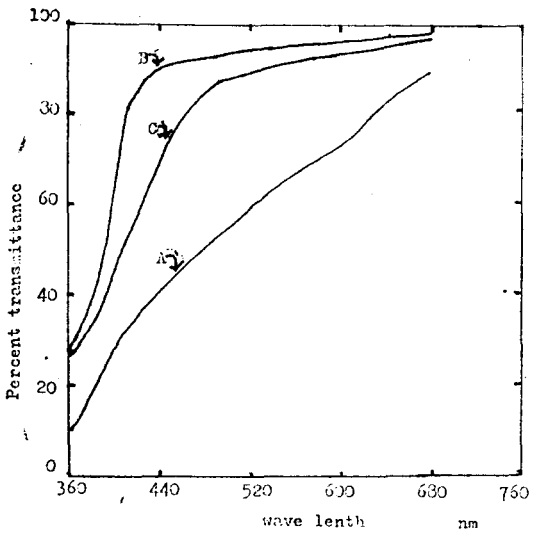

Fig. 7. Spectral transmittance curves for alkaline solutions of protein isolates;A, B and C.

for protein isolates, A and Cindicate that considerable pigment was retained in the pretein, whereas protein isolate at pH 5.6 show only slight pigmentation.

Nitrogen solubility. The minimum nitrogen solubility of isolates, A, B and C was lowest at pH 5.6 and the solubility increased rapidly above pH 7 to 80% at pH 9.1. The solubility alse increase rapidly below pH and reached 79% at pH 2.2.

Amino acid composition. Table 4 shows the amino acid composition of the isolates. The isolates had a very well balanced amino acid pattern, illustrated by

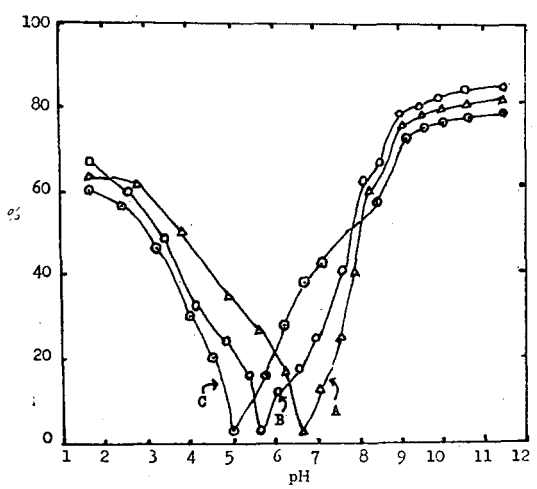

Fig. 8. Nitrogen solubility curve of rapeseed protein isolates; A, B and C.

Table 4. Amino acid composition of the isolates (mg amino acid/g isolate on as is basis)

Amino acid	Isolate		
	A	B	C
Arg	56.5	59.1	56.0
Lys	37.9	45.1	44.9
His	24.1	23.5	23.1
Asp	54.0	57.0	48.9
Glu	190.2	141.0	154.0
Cys	17.2	14.2	21.8
Met	18.1	18.2	18.0
Ile	31.9	36.6	33.9
Leu	62.5	75.2	61.0
Tyr	15.5	30.0	2415
Phe	32.8	33.9	34.4
Pre	70.2	61.2	69.0
Gly	39.0	41.9	39.3
Ala	34.5	38.5	34.8
Ser	37.9	41.8	37.0
Thr	29.2	38.8	33.1
Val	40.1	40.1	41.0
NH3	25.4	20.0	13.4
Chemical score	81	100	100

their high chemical score, which for the B and C isolates were calculated to be 100. For isolate A lysine was the first limiting amino acid closely followed by tyrosine. The fact that the content of cystein was high and that no trace of lysinoalanin was found in the isolates, indicate that detrimental changes in the nutritive value of the proteins were not produced during the extraction of the rapeseed meal at pH 11.0.

Electrophoresis. The patterns of distribution of various polypeptides in the protein isolates A, B and C from rapeseed, as determined by sodium dodecyl sulfate gel electrophoresis, are shown in Figure 9. It is evident that protein isolates A, B and C are distinctly different with regard to the composition of polypeptides. As indicated in Fig. 9 most of the acid and neutral proteins were contained in the isolate A, and isolates, B and C contained mainly basic protein fractions.

Isoelectric focusing. A pH range of 3 to 10 for electrofocusing enabled us to obtain a complete isoelec spectrum of both basic and acidic proteins (Fig. 10).

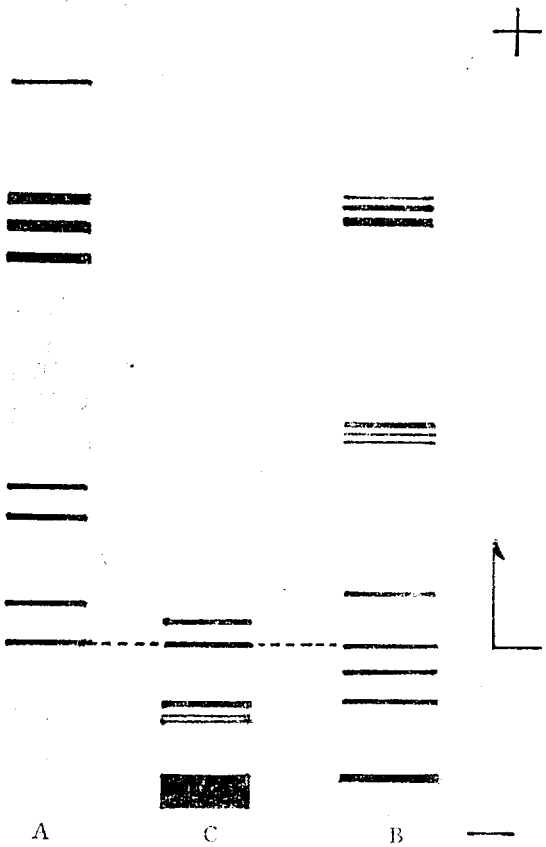

Fig. 9. Gel electrophoretic pattern of the protein isolates obtained at pH 6.7, 5.6 and 5.0.

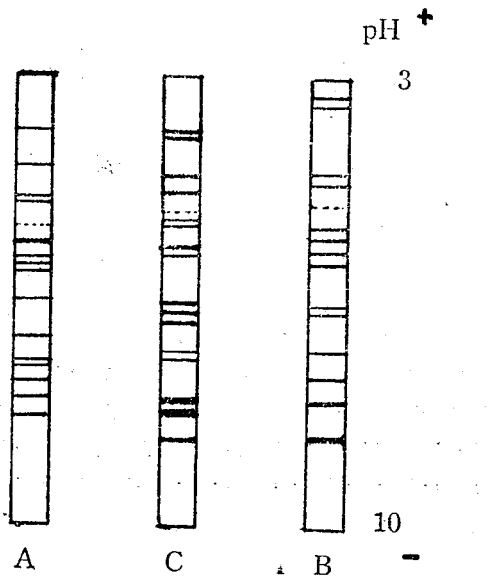

Fig. 10. Disc isoelectric focusing in a pH range of 3 to 10 of the protein isolates A, B and C.

It was observed that the majority of the protein isolates have isoelecctric points within the range of pH 4.5 to 10. Isolate A was found to be especially rich in acidic proteins (IP 4.5-6). This is consistent with the presence of large amount of high molecular weight proteins in isolate A and fact that these proteins are to have isoelctric points in the 4-7[24], isolate B only contained proteins with intermediate and low molecular weights.

Molecular sieve chromatography. Figure 11 shows the molecular sieve chromatogram obtained for the alkaline meal extract on the Sephadex column. Two distinct protein fractions, corresponding to the peaks at fraction 30 and 40 can be distinguished. Their molecular weights were calculated to be over 250,000 and around 150,000, respectively. This is in agreement with the findings of Bhatty *et al.*[25]. The third rather broad protein peak corresponds to two groups of proteins, one having molecular weights of about 50-75,000 and the other having molecular weights 13,000.[26].

Figure 12 represents molecular sieve chromatograms of samples obtained by dispersing the isolates in the pH 11 buffer, It should be noted that the isolates did not dissolve completely in the buffer. The fact that only small amounts of high molecular weight proteins occurred in the chromatogram Fig. 12A, thus indicates that the high molecular weight proteins in

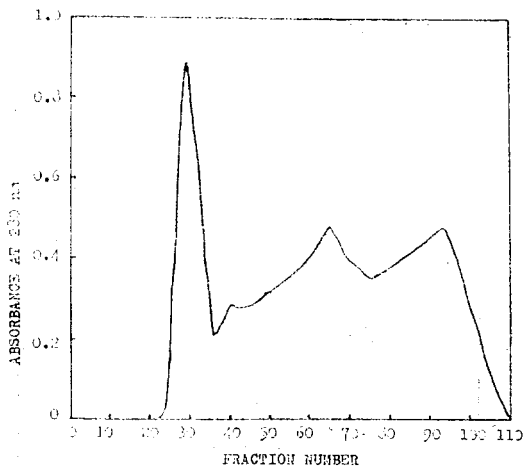

Fig. 11. Molecular sieve chromatogam of the rapeseed meal extract on Sephadex G-150 in 0.01m ammonium acetate/ammonia buffer of pH 11.0 containing 0.1m NaCl.

isolate A did not easily redissolve at pH 11. According to Fig. 12B isolate B gave a protein peak, which indicate the presence of high molecular weight proteins.

Figure 13 shows molecular sieve chromatograms of supernatant A, B and C. As can be seen from Fig. 13, all of the supernatants contained very little if any protein or other substances absorbing at 280 nm. This shows that rapeseed proteins can be quantitatably precitated either in a one step processor in a two step process using CMC or SA as a precipitation aid.

As seen from Fig. 13, all of supernatants contained no carbohydrates fraction 35 and higher. This indicates that all added CMC or SA was precipitated when isolates A, B and C were prepared.

In conclusion, The results presented shat the direct precipitation by acid of alkaline rapeseed extracts give protein isolates in rather low yields. The nitrogen recovery could be considerably improved. however. by conducting the precipitation step in the presence of sodium alginate.

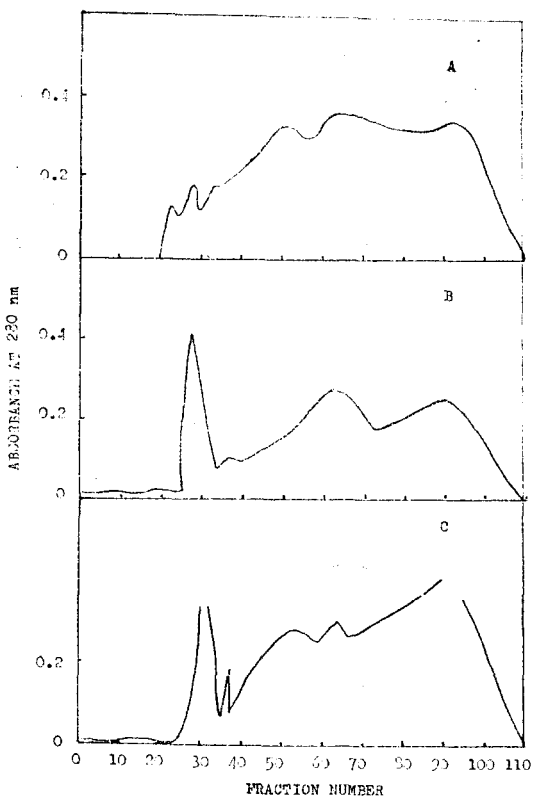

Fig. 12. Molecular sieve chromatogram of isolates A, B and C on Sephadex G-150 in the pH 11.0 buffer.

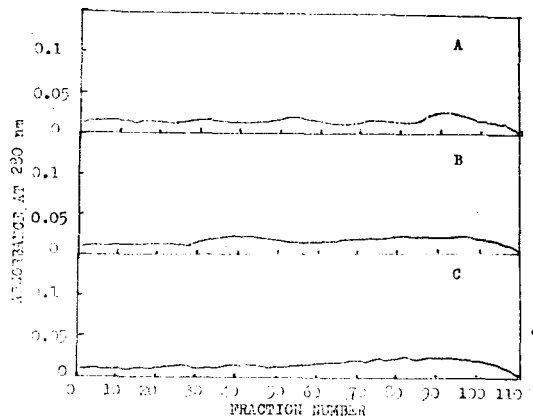

Fig. 13. Molecular sieve chromatogram of supernatant A′, B′ and C′ on Sephdex G-150 in the pH 11.0 buffer.

The pH of precipitation using maximum protein isolates was 6.7, 5.6 and 5.0. A countercurrent procedure was a prerequisite for a continuous large scale production of protein isolates.

The patterns of distribution of molecular weights were 13,000 to 250,000. The colors of protein isolates were improved by washing and freez-drying with acetone. and the proteins isolated were free of toxic factors as extracted by cold alkaline extractions.

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要 約

脫脂 油菜粕中에는 必須 아미노酸이 多數含有한 良質의 蛋白質을 含有하고 있으나 毒素인 myrosinase이 活性을 抑制시키거나 酵素 加水分解物인 isothiocyanate 및 oxazoolidinethione을 除去하여야 한다.

이와 같은 毒素를 myrosinase 活性을 減少시키는 方法으로 처리하여 全然無毒한 蛋白質를 分離하였다.

即 pH11.0에서 冷時에 抽出하고 0°C에서 沈澱을 分離하였으며 食品化學的인 性質 比較를 하였다.

油菜蛋白質은 分子數가 많아 沈澱時에는 助劑인 或은 알긴 酸소 다ㅡ 를 利用하여 좋은 結果를 얻었다.

또 pH 6.7, 5.6 및 5.0에 따라 色相을 달리하는 蛋白質이 分離되었고 水洗와 acetone을 使用하여 色素를 除去시킬 수 있었으며 冷凍乾燥하여 變色을 防止 하였다. 逆流 抽出法은 量産할 수 있는 蛋白質 抽出 方法이다.

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