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CHANGES OF PHYSICO-CHEMICAL PROPERTIES OF RAPESEED PROTEINS DURING THE TECHNOLOGICAL PROCESS OF PROTEIN CONCENTRATE PRODUCTION, PART I

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Key words: repeseed proteins, protein concentrate, proteins complexes, enzymatic hydrolysis

Investigations were made of the physical and chemical properties of rapeseed proteins and their changes during the technological process of protein concentrate production. The process of amino acid desamination and decarboxylation takes place during concentrate production. Albumin and globulin fractions form aggregates of the dimer and trimer type, and aggregates with nucleic acids and polyphenols. The albumin and globulin fractions of the protein concentrate are characterized by different solubility and susceptibility to enzymatic proteolysis than their respective fractions in rapeseed meal.

INTRODUCTION

Rapeseed proteins have the highest biological value of oil plant seeds [4, 17, 18] and, therefore, much attention has been devoted to them. MacKenze et al. [11] have noted that oil plant reserve proteins are characterized by a globular shape. They form a complex consisting of three fractions, with a sedimentation constant of 12s-7s-2s. The dissociation of this complex depends on the environmental pH. Studies by Goding et al. [8] showed that the protein complex stabilizing factor, with a sedimentation constant of 12s, was primarily hydrogen and not disulfide-bond. He also indicated that one of the polypeptides of fraction 12s was a glycoproteide. Bhatty et al. [2] distributed muriatic extract obtained from rapeseed on Sephandex gel and recorded that the fraction with a molecular weight over 10^5 daltons was a dimer consisting of a fraction with a sedimentation constant of 12s, and a fraction with 7s constant.

Physical and chemical properties of rapeseed proteins undoubtedly undergo some changes under the influence of technological processes of obtaining protein preparations, in this case of the concentrate. The aim of this paper is to determine the range of these changes.

MATERIALS AND METHODS

Rapeseed of the Skrzeszowicki variety was obtained from the Institute of Experimental Breeding and Plant Acclimatization in Poznań.

Rapeseed meal was obtained as follows: seeds were mixed with dry ice and ground in an impact mill in order to obtain fractions which would pass through a size 100 mesh sieve strainer. Grounded seeds were extracted with petroleum ether in a Soxlet apparatus.

Protein concentrate from rapeseed was obtained in the following way: whole seeds were subjected to water extraction (pH 9.0) at a temperature of 363 K for 30 min. Seeds swollen after water extraction were passed between rollers in order to remove the hulls. The mixture of hulls and cotyledons was rinsed with water at a temperature of 353 K for 30 min. After rising, the mixture was dried and hulls were separated with an air stream. Fat was removed from the cotyledons with petroleum ether after pulverization. The concentrate used in the experiment passed through a size 100 mesh strainer.

The meal and the concentrate of rapeseed protein were subjected to the following procedures:

1. Protein fractionation according to Osborn method [19]: 100 g of meal or concentrate were extracted successively with water, 0.5% sodium chloride, 70% ethanol and 0.2% sodium hydroxide, at a temperature of 278 K. Particular extractions were carried out until the disappearance of absorption in UV, at 280 nm. Then the factions were dialysed in the presence of redistilled water, at a temperature of 278 K, and condensed using Sephadex G-25 gel. The obtained protein fractions were lyophilized and stored untill determinations. Nitrogen content was determined by Kjeldahl method.

2. Determination of amino acid composition [15]: samples were hydrolized in nitrogen atmosphere of 6 M HCl in a temperature of 383 K for 21 hours. Amino acids were determined by an automatic amino acid analyser produced by Jeol.

3. Filtration was conducted on Sephadex gels G-100 and G-200. The albumin and globulin fractions were separated on 1.5×100 cm columns, with water as an eluent for the albumin fraction, and 0.9% NaCl for the globulin fraction. 2 cm^3 fractions were collected and the optical density was measured at 280 nm. 20 mg of protein were placed on the columns.

4. Molecular weights of particular albumin and globulin fractions (obtained after separation on gel Sephadex G-200) were determined using the relationship given by Determan [5].

5. Electrophoresis on polyacrylamid gel. Gel was prepared according to Reisfeld et al. so as to obtain a 15% concentration. 50 μl of protein solution at concentration 10 mg/cm³ were placed on plates with the gel. Electrophoresis was carried out using an electrode buffer: glycine, 0.01 M acetic acid, water with pH 3.4 at voltage reduction of 30 V/cm. Protein fractions were dyed with Comasil Blue.

6. Determination of albumin and globulin solubility depending on environmental pH. Mixing of basic solutions at proper proportions made it possible to obtain buffer solutions of pH value from 2.2 to 8.0. Suitable environmental pH was obtained with the use of a citric buffer [16]. 4 mg protein were added to 4 cm³ of the buffer solutions and shaken for 15 min. Then the solutions were centrifuged at 3000 g. 2 cm³ of liquid from above the precipitate were taken for spectrophotometric analysis. Extinction was measured at a wave length of 280 nm.

7. Enzymatic hydrolysis with pepsin and trypsin. For protein hydrolysis the following enzyme solutions were used: 2 mg pepsin (BDH) were dissolved in 10 cm³ of acetate buffer, at a pH of 2.0, and 2 mg trypsin were dissolved in 10 cm³ of tris buffer, at a pH of 7.4. 10 mg protein were dissolved in 5 cm³ buffer and the enzyme solution was added, at a rate of 1 cm³. The samples were incubated at 310 K for 1.5 hours. Then 2 cm³ of 10% TCA were added. The pricipitated protein was centrifuged at 2000 g. TCA was removed from the solution by ethyl ether extraction, until a neutral reaction was obtained. The neutralized solutions were evaporated to dryness and dissolved in 0.5 cm³ of redistilled water. 80 μl of the solution were spread on blotting paper for electrophoretic analysis [12].

RESULTS AND DISCUSSION

A CHARACTERISTIC OF RAPESEED AND CONCENTRATE PROTEIN SOLUBILITY ACCORDING TO OSBORN

Total nitrogen in the rapeseed meal amounted to 6.4%, and to 8.7% in the concentrate. An analysis of data shown in Tab. 1 shows that the content of nitrogen soluble in water was 2.8 times lower, and of that soluble in 5% NaCl approximately 2.6 times lower in the concentrate than in rapeseed meal. However, in the concentrate the content of nitrogen soluble in 70% ethanol was 6.9 times higher, and of that soluble in 0.2% solution NaOH — approximately 4.6 times higher. The residue content of nitrogen occurred at similar levels in the rapeseed meal and in the con-

Table 1. Soluble nitrogen of the rapeseed meal and rapeseed protein concentrate; in per-cent of total nitrogen

	H_2O	5% NaCl	70% C_2H_5OH	0.2% NaOH	N-residual
Rapeseed meal	42.6	25.0	2.6	7.0	22.7
Concentrate	15.3	9.6	17.2	32.4	25.5

centrate. Substantial changes were noted in the nitrogen content extracted with water (albumins) and 5.0% NaCl (globulins). There was a marked reduction of albumin and globulin content in the concentrate compared to the rapeseed protein, and a distinct increase of protein soluble in ethanol and NaOH solution. This fact proves that part of the albumin and globulin fractions underwent denaturation.

AMINOACID COMPOSITION

Tab. 2 presente the amino acid composition of rapeseed meal, protein concentrate, and fractions obtained according to Osborn. In all fractions except albumins a lower recovery rate of amino acids was noted in the concentrate than in the rapeseed meal. The highest decrease of amino acids was recorded in the globulin fraction of the concentrate (6.6%). On the other hand, the increase of amino acides in the albumin fraction of the concentrate (8.2%) was caused by a higher recovery of amino acid nitrogen. The increase of amino acid nitrogen recovery was probably caused by a decrease of non-protein components in the concentrate, such as nucleic acids and phenolic compounds [3, 10]. Considerable differences observed in the amounts of amino acid nitrogen in albumin and globulin fractions prove that these proteins undergo degradation and conformation changes during the technological process. The marked increase of ammonia content and a decrease of acidic amino acids in the concentrate suggest that desamination and decarboxylation takes place during the technological process.

SEPARATION ON THE SEPHADEX GELS G-100 AND G-200

Separation of albumin and globulin fractions on Sephadex gels G-100 and G-200 is shown in Fig. 1 and 2. As a result of separation of the rapeseed and the concentrate albumin fractions on Sephadex gel G-100, fractions of similar elution volumes (Ve) but of different absorption values at 280 nm were obtained. This may suggest that structural changes

Table 2. Amino acid composition of the rapeseed meal, rapessed protein concentrate and of the individual fractions by Osborn (g/100 g of protein)

Amino acid	Rapeseed	Concentrate	*)%	Soluble protein in											
				H_2O			5% NaCl			70% C_2H_5OH			0.2% NaOH		
				R	C	*)%	R	C	*)%	R	C	×%	R	C	*)%
Ala	4.5	4.1	-7.9	4.8	5.6	+16.6	4.2	3.7	-12.0	4.1	3.4	-17.1	5.9	4.2	-28.8
Arg	5.7	5.7		5.9	6.8	+15.2	5.6	6.6	-17.8	3.5	2.4	-31.4	trace	4.5	
Asp	6.9	6.1	-11.6	5.8	7.1	+22.4	4.6	4.2	-8.7	7.8	7.6	-2.6	9.9	6.1	-38.4
Cys	2.3	2.2	-4.4	2.0	1.8	-10.0	0.4	0.3	-25.0	—	—		—	—	
Glu	17.9	16.5	-7.8	20.0	17.9	-10.5	20.1	14.8	26.4	14.5	16.1	+11.0	20.3	23.6	+16.2
Gly	4.8	4.5	-6.3	5.3	5.8	+9.4	4.2	4.6	+9.5	5.7	4.9	-14.1	6.5	4.7	-27.7
His	2.9	2.9		2.9	3.4	+17.2	4.0	4.0		2.6	2.6	-	2.0	1.9	-5.0
Ile	3.8	3.7	-2.7	2.6	3.7	+42.6	2.1	2.2	+4.8	2.0	1.8	-10.0	5.5	2.7	-51.0
Leu	6.1	6.0	-1.7	5.6	6.8	+21.4	7.2	7.3	+1.4	4.2	4.3	+2.4	6.1	6.0	-1.7
Lys	6.0	5.8	-3.4	7.0	6.4	-8.6	8.0	8.4	+5.0	7.9	7.6	-3.8	7.3	5.1	-30.2
Met	1.6	1.5	-6.3	0.7	0.3	-42.8	0.7	0.9	+28.6	0.9	0.9	-	1.8	1.2	-43.4
Phe	3.5	3.6	+2.8	3.5	4.1	+17.1	3.1	3.3	+6.4	2.1	1.8	-14.3	3.3	3.3	
Pro	9.6	10.1	+5.2	10.3	8.7	-15.6	12.5	10.5	-16.0	6.3	6.3	-	7.4	11.0	+48.6
Ser	4.2	4.3	+2.4	3.4	4.9	+44.1	4.2	4.6	+9.5	4.7	4.7	-2.2	5.2	4.2	-19.3
Thr	4.0	3.7	-7.5	4.3	6.4	+48.8	3.7	4.1	+10.8	6.3	5.8	-8.0	5.1	3.8	-25.5
Tyr	2.5	2.4	-4.0	1.7	2.7	+58.8	1.3	trace		2.4	2.4	-	trace	trace	
Val	4.3	4.5	+4.6	2.7	4.3	+59.2	4.4	4.2	-4.6	3.7	3.1	-16.3	3.7	4.3	+16.2
NH_3	1.8	5.3	+19.4	2.4	1.8	-25.0	2.3	4.5	+82.6	8.4	11.3	+34.0	6.0	8.0	+33.3
% recovery	92.4	92.9		90.9	98.5		92.6	88.2		87.1	86.2		96.2	94.8	
without NH_3	90.6	87.6		88.5	96.7		90.3	83.7		78.7	74.9		90.2	86.8	

*% percentage differente between content of amino acids in the rapesseed meal and rapeseed protein concentrate
- higher percentage of amino acids in the rapeseed than in the concentrate
+ higher percentage of amino acids in the concentrate than in the rapeseed

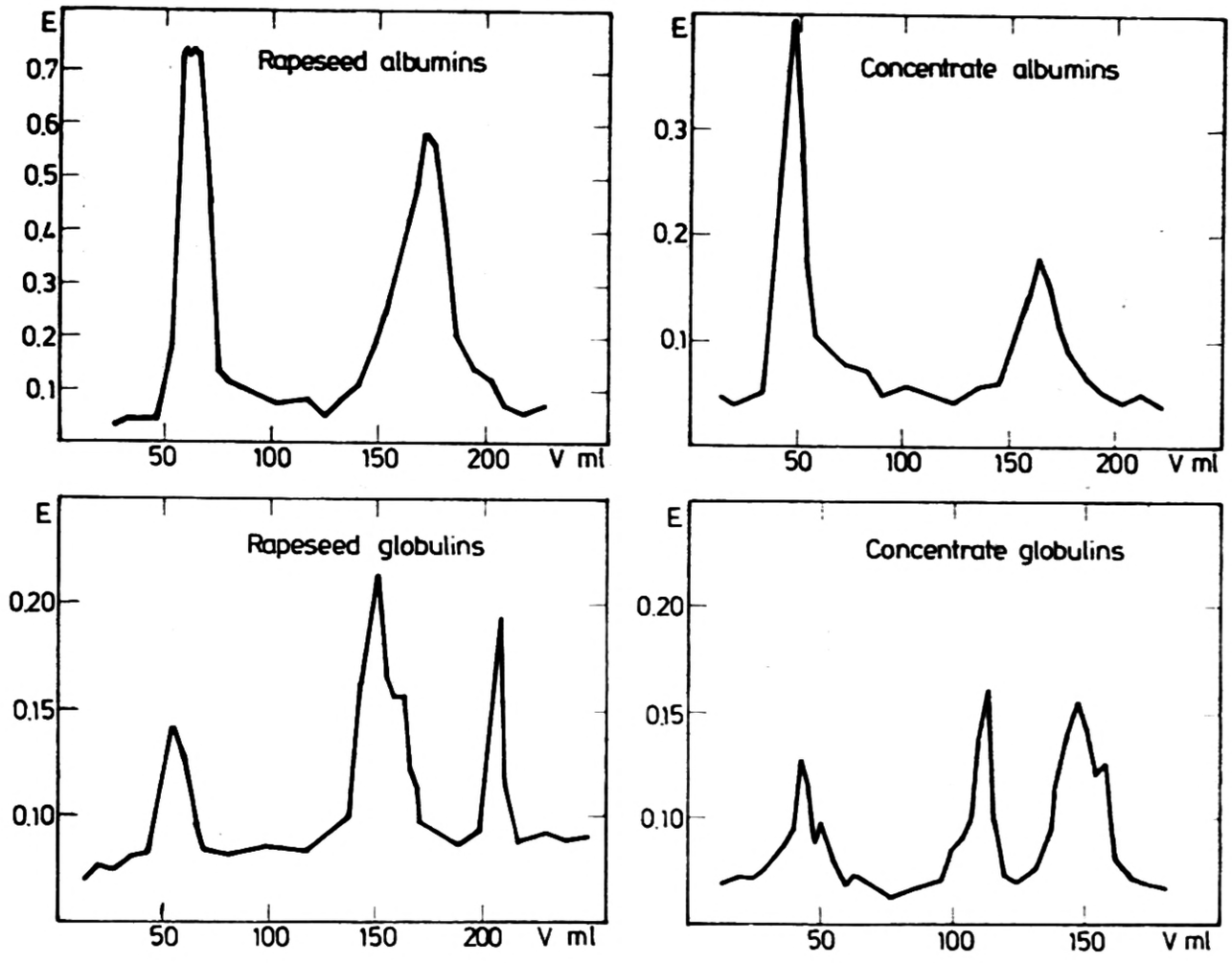

Fig. 1. Separation of rapeseed and concentrate albumins and globulins on Sephadex G-100

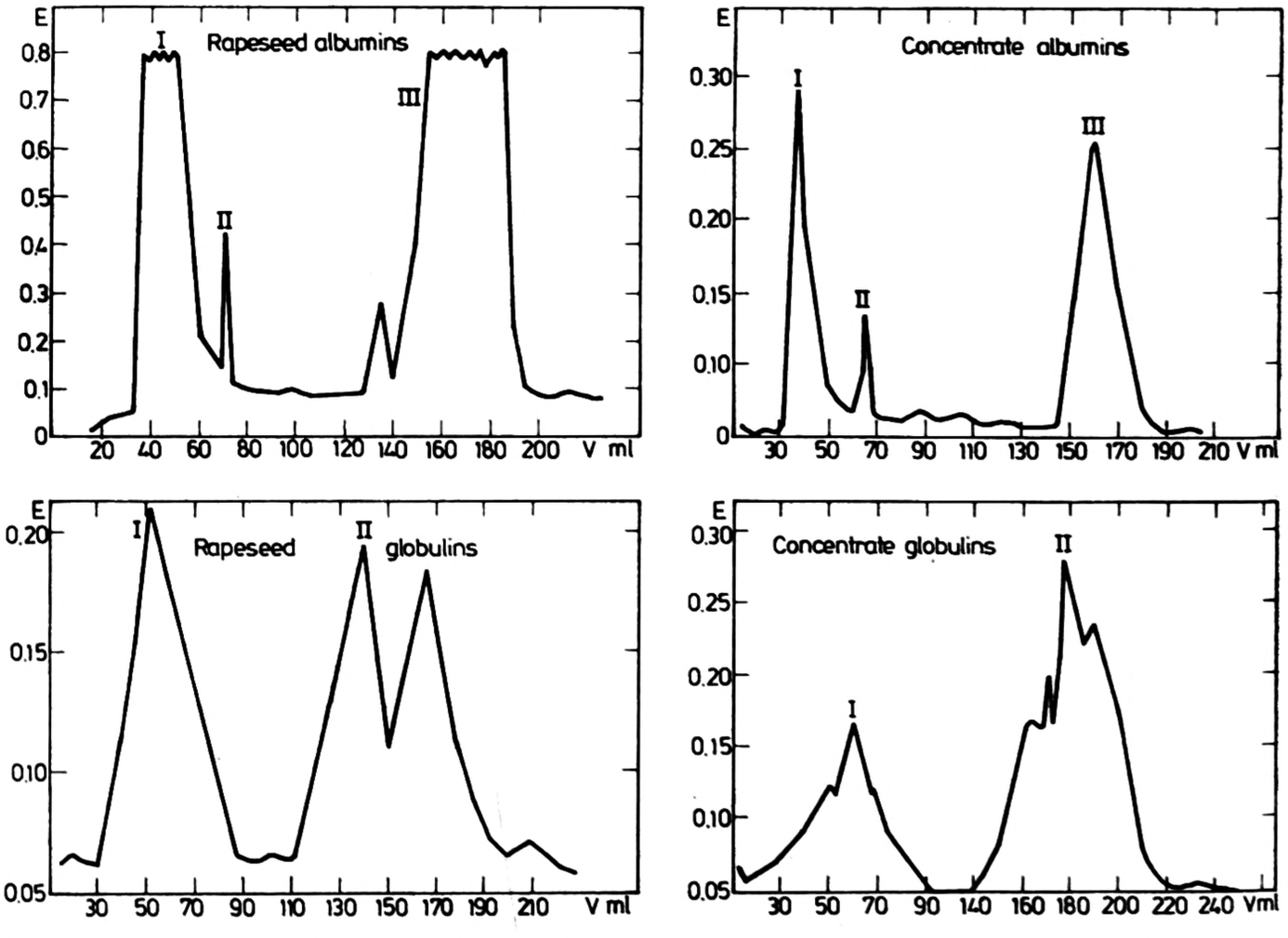

Fig. 2. Separation of rapeseed and concentrate albumins and globulins on Sephadex G-200

occurred in the albumin fraction of the concentrate, which caused blocking of tyrosine and tryptophane groups responsible for absorption of the UV radiation. On the other hand, fractions the Ve value lower than 50 cm^3, and also large fractions of Ve about 110 cm^3 appeared in the concentrate globulins while fractions of Ve about 200 cm^3 disappeared. It is possible that in case of the globulin fraction of the concentrate, processes of association or dissociation of protein complexes took place. The shape of the elution curves also suggests this possibility. Molecular weights presented in Table 3 also indicate that the albumin and globulin fractions

Table 3. Molecular weights of albumin anf globulin fractions of the rapeseed and rapeseed protein concentrate (in daltons)

Number of fraction	Rapeseed		Concentrate	
	albumins	globulins	albuminis	albuminis
I	191000±5000	296000±5000	212000±5000	32000±50000
II	35000±5000	10000	46000±5000	10000
III	10000	10000	100000	—

may from complexes of a dimer or a trimer type, or bindings with nucleic acids and polyphenols [3]. Differences in molecular weights between the albumin and globulin fractions of the rapeseed and the concentrate can also be attributed to a different spatial structure of protein complexes. This, in turn, could affect the Ve value which constituted a basis for the determination of molecular weights. These results are confirmed by other authors [1, 6, 7, 11, 14].

ELECTROPHORESIS

Electrophoretic investigations carried out by other authors on rapeseed proteins extracted by different solvents indicated the presence of various fractions [2, 7, 9].

For example, the so called globulin 12s protein complex was divided into six fractions, and the aqueous rapeseed extract into seven fractions [8]. Electrophoretic divisions presented in Fig. 3 show that rapeseed albumins separated into four fractions, these results being similar to those on Sephadex G-200. Electrophoretic studies on concentrate albumins confirmed the divisions on gel Sephadex G-200, i.e. the disappearance of one fraction compared with rapeseed albumins. Electrophoretic divisions of

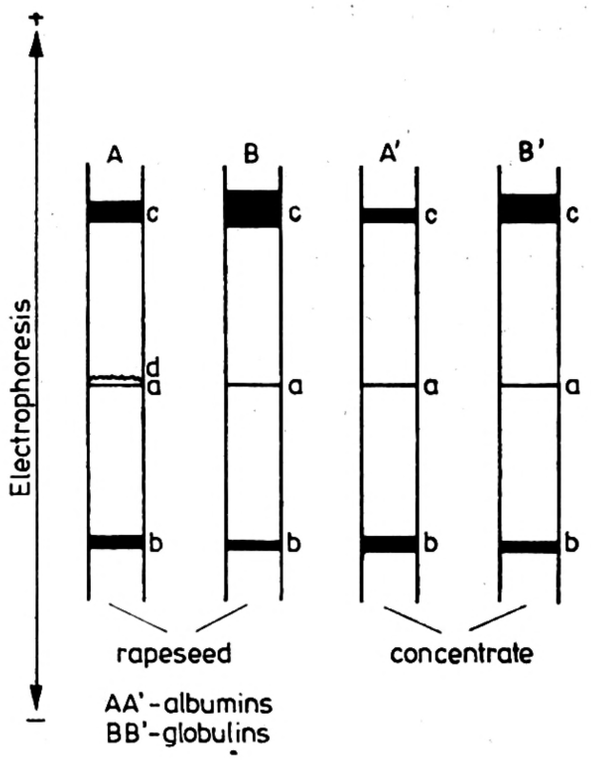

Fig. 3. Electrophorogram of rapeseed and concentrate proteins

globulins showed the presence of only three fractions, but fraction „C" appeared in larger quantities. These divisions complied with those obtained using gel Sephadex G-200.

SOLUBILITY OF ALBUMINS AND GLOBULINS DEPENDING ON THE ENVIRONMENTAL pH

Diagrams presenting the solubility of rapeseed and concentrate albumins and globulins are given in Fig. 4 and 5. Rapeseed albumins were most soluble at pH 6.0, and least soluble at pH 4.0. Concentrate albumins

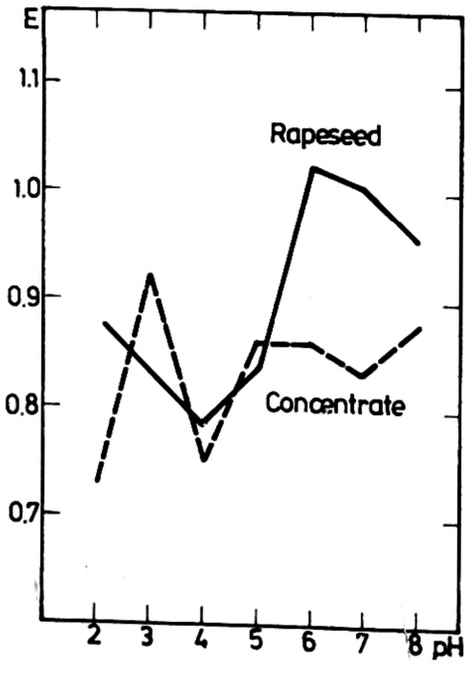

Fig. 4. Solubility of rapeseed and concentrate albumins depending on pH

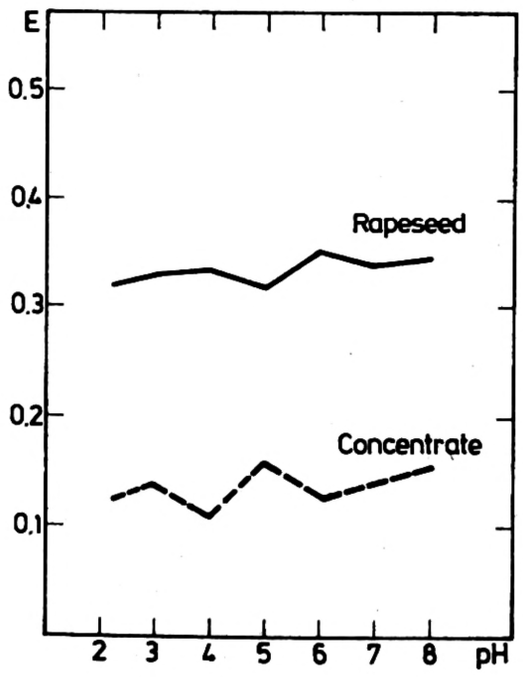

Fig. 5. Solubility of rapeseed and concentrate globulins

were most soluble at pH 3.0 and least soluble at pH 2.0 and 4.0. Rapeseed globulins were most soluble at pH 6.0 and least soluble at pH 5.0. Concentrate globulins were most soluble at pH 5 and least soluble at pH 4. Rapeseed albumins and globulins were more soluble than those in the concentrate. Differences in pH values for maximum and minimum solubility of albumins and globulins suggest that the ion character of rapeseed and concentrate proteins is different. This is supported by the differences in conformation structure caused by the technological process.

ENZYMATIC HYDROLYSIS

Enzymatic hydrolysis was carried out in order to determine changes of protein susceptibility to proteolytic enzymes during the technological process. Electrophoretic divisions of acidic and alkaline peptides are shown in Fig. 6, and the sum of all peptides — in Fig. 7, 8 and 9. Differences in

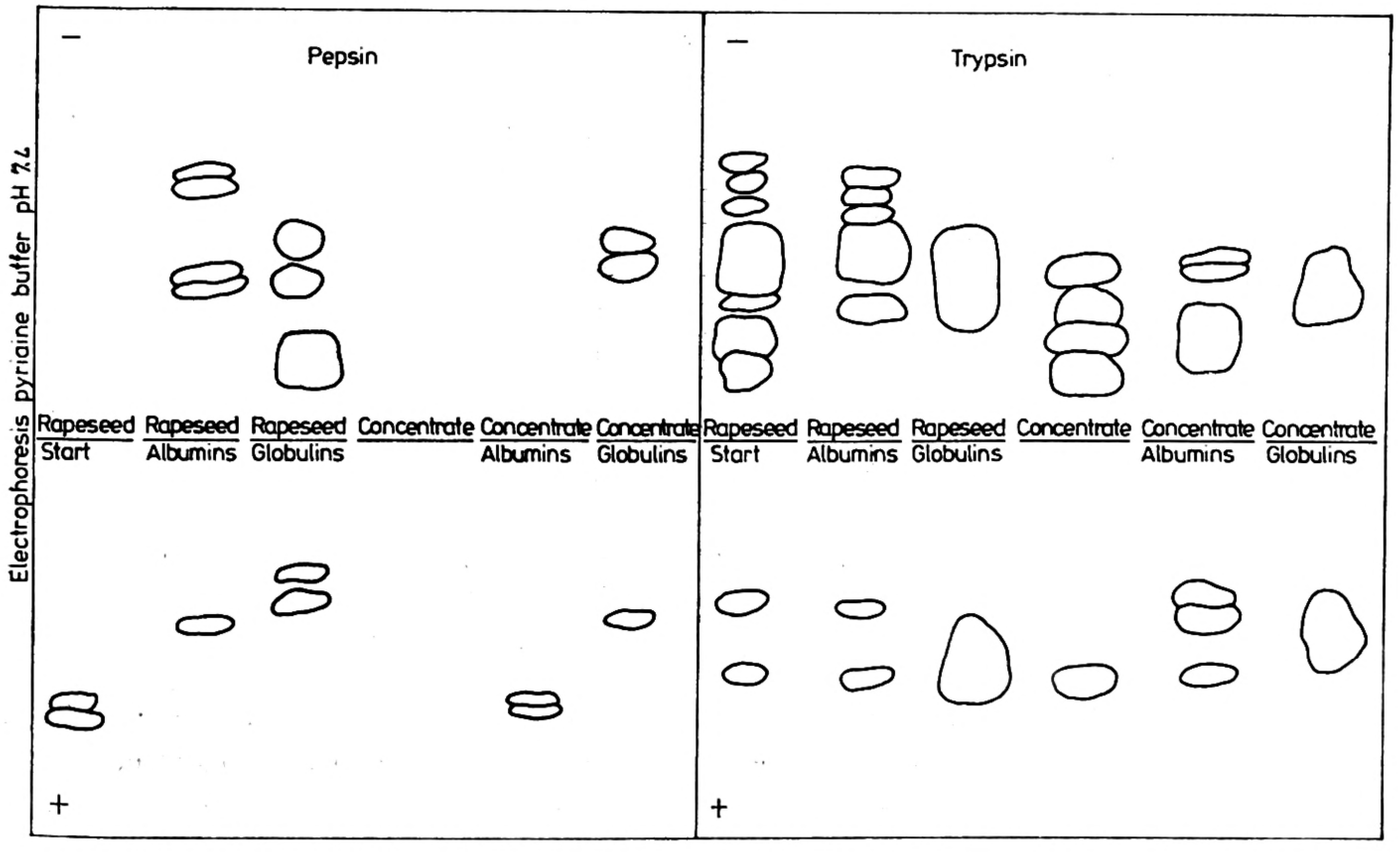

Fig. 6. Electrochromatogram of acid and alkaline peptides liberated by pepsin and trypsin from rapeseed meal, rapeseed protein concentrate, albumins and globulins

the releasing of peptides by pepsin and trypsin point to the dual character of conformation changes which make that in one case the protein susceptibility to enzymes decreased, and in another it increased.

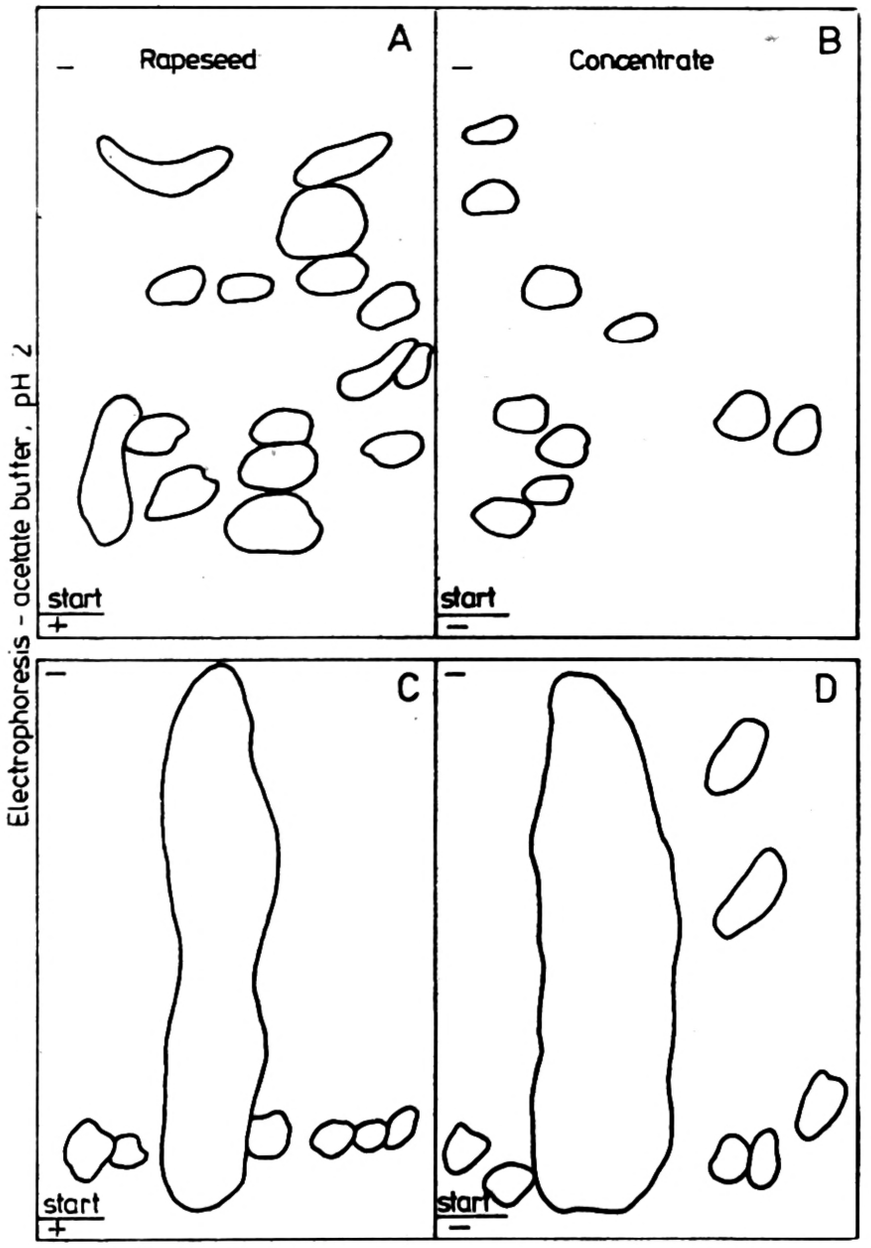

Fig. 7. Electrochromatogram of peptides liberated by pepsin and trypsin from rapeseed globulins and concentrate globulins; A and B — pepsin hydrolyzate, C and D — trypsin hydrolyzate

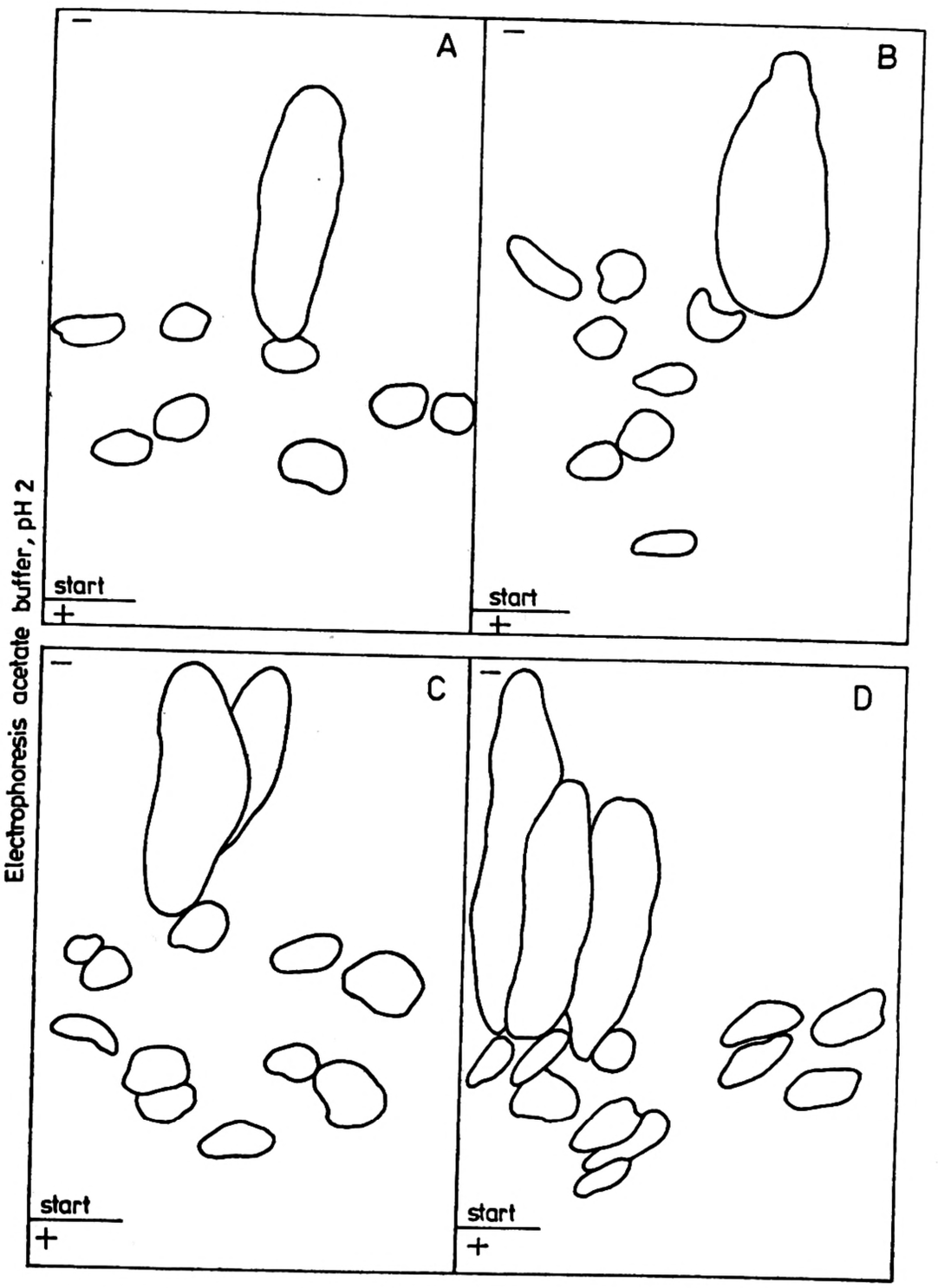

Fig. 8. Electrochromatogram of peptides liberated with trypsin from: A — rapeseed meal, B — concentrate, C — rapeseed albumins, D — concentrate albumins

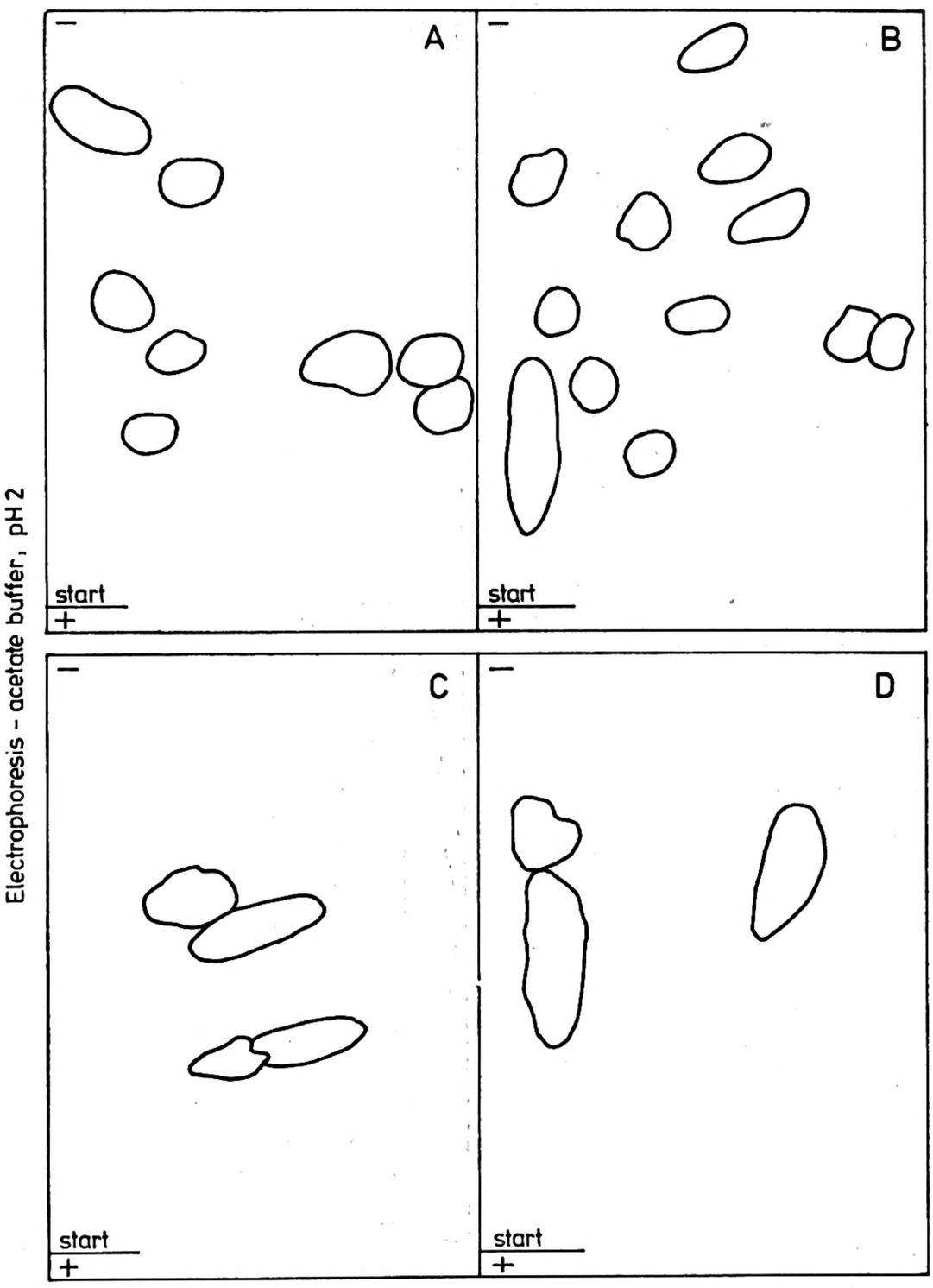

Fig. 9. Electrochromatogram of peptides liberated with pepsin from: A — rapeseed meal, B — concentrate, C — rapeseed albumins, D — concentrate albumins

CONCLUSIONS

1. A marked decrease in the content of the albumin and globulin fractions was noted in the concentrate compared to rapeseed meal protein, as well as a distinct increase of proteins soluble in ethanol and sodium hydroxide solution. This proves that during concentrate production part of the proteins undergoes a denaturation process.

2. Analysis of the amino acid composition showed that during concentrate production processes of amino acid desamination and decarboxylation take place. This means that the process of concentrate production causes partial hydrolysis of protein and further conversions of the released amino acids.

3. Solubility of the albumin and globulin fraction changes during the technological process of concentrate production. This fact points to the different ionic character of proteins in the rapeseed and the concentrate.

4. The concentrate protein is characterized by a different susceptibility to proteolysis than the rapeseed meal protein.

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ZMIANY WŁAŚCIWOŚCI FIZYKOCHEMICZNYCH BIAŁEK RZEPAKU W PROCESIE TECHNOLOGICZNYM OTRZYMYWANIA KONCENTRATU, CZ. I

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Streszczenie

W pracy badano fizyczne i chemiczne właściwości białek nasion rzepaku podczas technologicznego procesu otrzymywania koncentratu białkowego. Mączkę i koncentrat nasion rzepaku poddano następującym analizom: ekstrakcji metodą Osborna, określeniu składu aminokwasowego, sączeniu molekularnemu na żelu Sephadex G-100 i G-200, określeniu masy cząsteczkowej, elektroforezie na żelu poliakrylamidowym, określeniu rozpuszczalności białek frakcji albumin i globulin w zależności od pH hydrolizie pepsyną i trypsyną.

W koncentracie białkowym, w porównaniu z mączką rzepakową, stwierdzono obniżenie się zawartości białek w frakcji albumin i globulin oraz ich wzrost w frakcjach rozpuszczalnych w etanolu i 0,2 M NaOH. We wszystkich frakcjach, za wyjątkiem albumin, stwierdzono mniejszy odzysk aminokwasów w koncentracie niż w śrucie rzepakowej. Największy ubytek aminokwasów zaobserwowano w frakcji globulin koncentratu. Znaczny przyrost zawartości amoniaku i zmniejszenie się zawartości aminokwasów kwaśnych w koncentracie dowodzi o zachodzącej w czasie procesu technologicznego dezaminacji i dekarboksylacji. W wyniku rozdziału frakcji albumin nasion rzepaku i koncentratu na żelu Sephadex G-100 uzyskano frakcje o podobnych objętościach elucji i zakresie mas cząsteczkowych od 10-200 tys. Natomiast w przypadku globulin koncentratu pojawiły się frakcje o odmiennych wartościach objętości elucji. Masy cząsteczkowe tych frakcji zawarte były w granicach od 10-320 tys. W wyniku rozdziałów elektroforetycznych albumin nasion rzepaku rozdzieliły się na cztery frakcje, podczas gdy albuminy koncentratu tylko na trzy. Elektroforetyczne rozdziały globulin nasion rzepaku i koncentratu w obu przypadkach wykazały obecność trzech frakcji. Wyniki te były zgodne z rozdziałami na żelu Sephadex G-200. Albuminy i globuliny nasion rzepaku były lepiej rozpuszczalne niż koncentratu. Albuminy nasion rzepaku najlepiej rozpuszczały się w pH 6,0, a najsłabiej w pH 4,0. Natomiast albuminy koncentratu największą rozpuszczalność wykazywały w pH 3,0, a najmniejszą w pH 2,0 i 4,0. Globuliny nasion rzepaku najlepiej rozpuszczały się w pH 5,0, a najsłabiej w pH 6,0. Natomiast globuliny koncentratu najsłabiej rozpuszczały się w pH 4,0, a najlepiej w pH 5,0. Hydroliza frakcji białkowych nasion rzepaku i koncentratu za pomocą pepsyny i trypsyny dowiodła istotnych różnic w konformacji badanych frakcji. Fakt ten przejawiał się w uwalnianiu różnych ilości peptydów przez zastosowane enzymy.