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

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Key words: protein complexes, protein conformation, UV and IR spectroscopy, circular dichroism.

The work presents a characteristic of some elements of the protein conformation of albumin and globulin fractions isolated from rapeseed meal and rapeseed protein concentrate (see Part I). Investigations were based on spectrophotometry in the ultraviolet and infrared radiation, and on circular dichroism. The proportion of alpha, beta and gamma structures was determined in rapeseed and concentrate albumin and globulin fractions.

INTRODUCTION

The first part of this work gives a characteristic of changes taking place in rapeseed protein during the production of rapeseed protein concentrate. A decrease of albumins and globulins, with a simultaneous increase of proteins soluble in ethanol and 0.2 M NaOH, was observed in the concentrate. The technological process caused a deamination and decarboxylation of some aminoacids. The solubility of the albumin and globulin concentrate and susceptibility to proteolysis were also changed. The results suggest that the proteins of rapeseed meal and rapeseed protein concentrate are forming different complexes with non-protein compounds, probably of the dimeric and trimeric types.

This part of the work presents a characteristic of some conformation elements of the albumin and globulin fractions isolated from rapeseed meal and rapeseed protein concentrate.

MATERIALS AND METHODS

The albumin and globulin fractions of rapeseed meal and concentrate were obtained as described in Part I. The following studies were carried out:

1. Characteristic of the ultraviolet spectra. Absorption spectra in the UV of the albumin anf globulin fractions before and after heating with formic acid were obtained with a spectrophotometer (Opton). Hydrolysis of the albumin and globulin fractions was conducted as follows: 1 cm^3 of concentrated formic acid was added to 5 mg protein and heated at 448 K for one hour. Then samples were evaporated to dryness at 313 K under reduced pressure. The dry residue was dissolved in water in case of albumins, and in 0.9% solution of sodium chloride in case of globulins [1].

2. Characteristic of the infrared spectra. The infrared spectra of albumin and globulin fractions were obtained with the pellet technique, using an IR-71 spectrophotometer (Carl Zeiss, Jena). The pellets were prepared by Mixing 2 mg protein with 800 mg KBr.

3. Circular dichroism. Measurements of the circular dichroism of the albumin and globulin fractions were carried out with the use of a Dichrograph Iobin Mark III. Protein concentrations ranged within 0.0128 to 0.0322 M/dm^3. The proportional content of the α, β and γ structure was calculated from a computer programme based on literature [5, 6, 11, 12]. Ribonuclease, lysozymes and myoglobulin were used as standards to determine the proportional content of particular structures. Calculations were carried on an Odra 1204 computer.

RESULTS AND DISCUSSION

Hydrolysis of the albumin and globulin fractions with formic acid was carried out in order to determine the presence of nucleic acid in these proteins. Ultraviolet spectra of the albumin anf globulin fractions of the rapeseed and the concentrate before and after hydrolysis with formic acid are presented in Fig. 1. A characteristic feature of the spectrum of albumin fraction in the concentrate was its specific shape (A), which was totally different from the typical bell-shaped curve of proteins. On the other hand, the spectrum of the rapeseed albumin fraction had an absorption maximum at 320 nm (A'). After hydrolysis with formic acid, the spectra of both rapeseed and concentrate fractions became bell-shaped (B, B'). Absorption maxima of these spectra appeared at 280 nm and had higher values. Spectra of rapeseed and concentrate globulin absorption had their maxima at 270 nm (A, A'). After hydrolysis with formic acid, globulin spectra became bell-shaped, with their absorption maximum at 280 nm

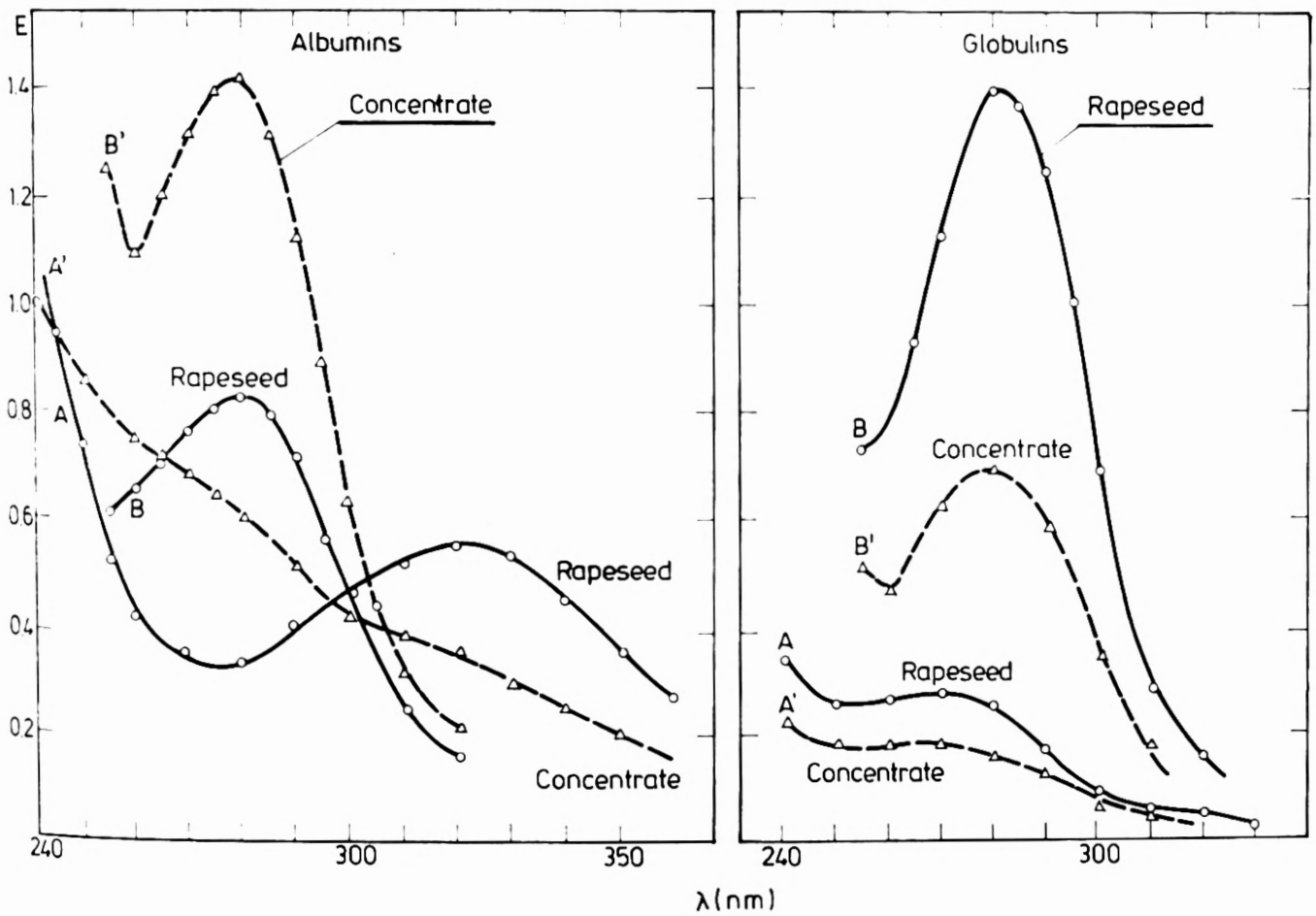

Fig. 1. UV absorption spectra of albumins and globulins of rapeseed and rapeseed protein concentrate, before and after hydrolysis with formic acid. A and A' — before hydrolysis, B and B' — after hydrolysis

(B, B'). The extinction value at this wave length was 3.5 times higher than the value at 270 nm. In this context, the spectrum of the rapeseed albumin fraction was of particular interest. Appearance of an absorption maximum at 320 nm can be explained by the presence of phenolic acids associated with proteins [7, 10]. Some phenolic acids are characterized by two absorption bands within the UV range [10]. These are: gentisic, caffeic and ferulic acids, for which a second absorption band appears within the range of 317-335 nm. It can be assumed that when rapeseed albumin fractions were heated with formic acid, some phenolic acids were released and underwent isomerization. This caused a displacement of absorption maximum to shoreter waves, of about 280 nm.

Probably the increase of absorption resulted from an exposure of tyrosine and tryptophane protein groups. Increase of absorption at 260 and 280 nm, observed in case of the rapeseed globulin fraction after heating with formic acid, can be explained by the presence of nucleic acids associated with this fraction [1]. Conditions during heating of protein with formic acid were sufficient for a hydrolysis of nuleci acids [9]. Hydrolysis of nucleic acids resulted in the appearance of a hyperchromic effect, consisting in the fact that the sum of extinction of nucleotide components was greater than the extinction of polynucleotides [4]. These results point

to evident differences between the proteins of rapeseed and of the concentrate.

Infrared spectra of the rapeseed and concentrate albumin and globulin fractions are given in Fig. 2. Characteristics of absorption bands and va-

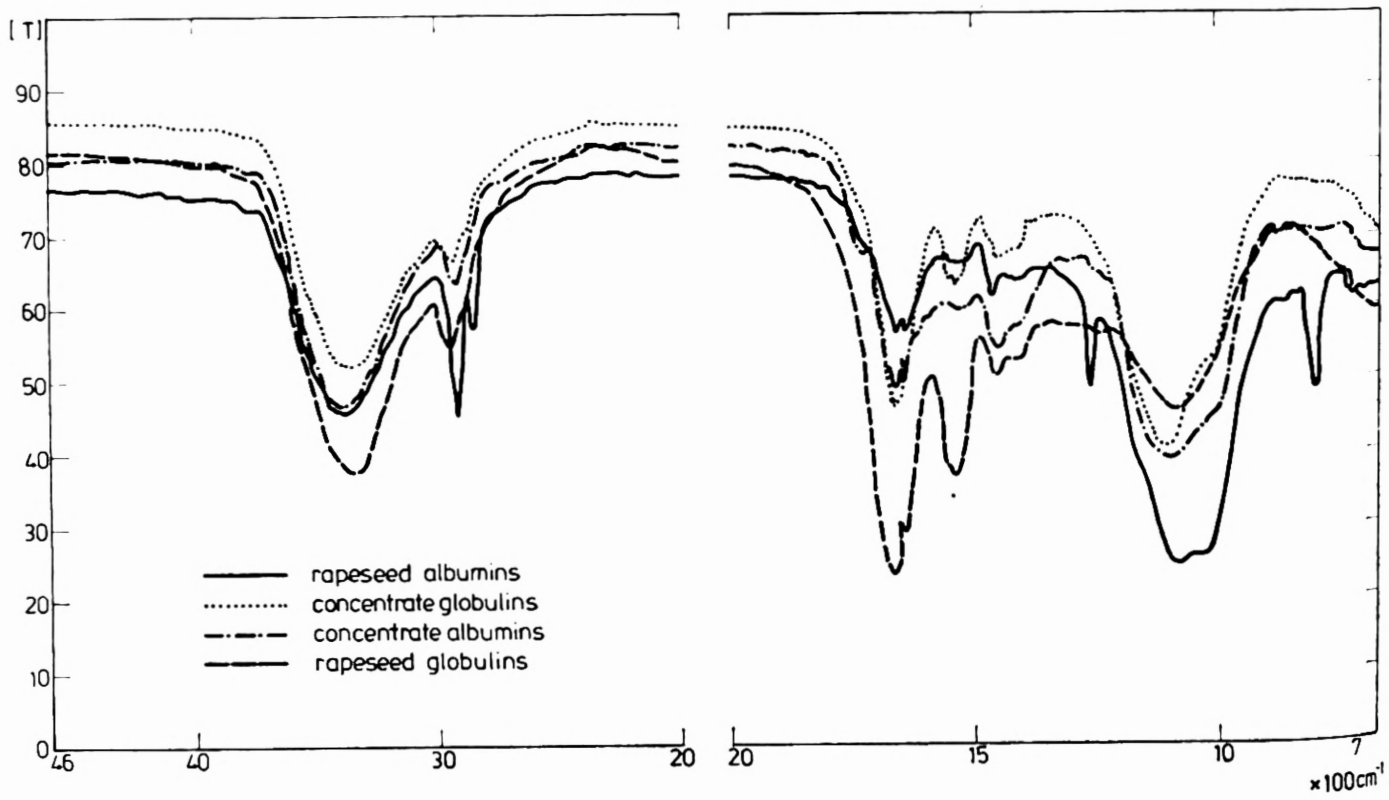

Fig. 2. IR spectra of rapeseed and concentrate proteins

lues of wave numbers are presented in Table 1. Seven absorption bands occurred in the spectra of albumin and globulin fractions of the rapeseed. The albumins of the concentrate featured five absorption bands, and the globulins — seven. Comparing the spectra of albumin and globulin fractions it was noted that the differences occurring in the absorption bands 1640-1660 and 1530 cm^{-1} were the most interesting as regards the conformation structure. On the basis of these absorption bands, the proportional content of the structures was determined; it amounted to 14% for rapeseed and concentrate globulins [2, 8]. In rapeseed albumins this value amounted to 18%, and in concentrate albumins — to 17%. Analysis of the spectra showed the presence of non-protein compounds (probably phenolic acids) in rapeseed albumin fraction. Absorption bands 2920-2930 cm^{-1} (methylene group) connected to the benzene ring, 3400 cm^{-1} of group C-O and OH, 770-800 cm^{-1}, distributed derivative, 1240 and 1100-1130 cm^{-1} could be attributed to the presence of nucleic acids (Table 1). This confirm the results obtained in UV. The above described absorption bands did not occur in the albumin and globulin fraction of the concentrate. This fact may result from lower concentration or lack of non-protein compounds in the concentrate. The remaining differences in the spectra resulted from the different conformation structure of proteins.

Table 1. Characteristics of absorption bands in the infrared spectra of the albumin and globulin fractions in rapeseed and concontrate

Number of absorption Bands cm^{-1}	Albumin		Globulin	
	rapeseed	concentrate	rapeseed	concentrate
1	770-800	—	—	—
	1.3 disubstituted derivative of benzene			
2	1010-1100	1010-1100	1000-1130	100-1130
	probably valent vibrations of the group ester type P-O-P			
3	1260	—	1240	—
	vibrations originating from the compounds of nucleic acids			
4	1400-1460	1400-1450	1400-1450	1400-1450
	vibrations of the Amid III type			
5	—	—	1530	1530
	vibrations of the Amid II type			
6	1640-1660	1640-1660	1640-1660	1640-1660
	vibrations of the Amid I type			
7	2920-2930	2920-2930	2930-2970	2930-2970
	asymetrical vibrations of CH_2 group and vibrations of the methyl group connected to the benzene nucleus			
	3400	3400	3200-3320	3200-3320
	vibrations of the Amid „A" type			

This is partly confirmed by the differences in the width of absorption bands in the range of 3200-3400 cm^{-1}.

Studies on circular dichroism of proteins enabled determination of the proportional share of particular structures α, β and γ [3]. The CD spectra of albumin and globulin fractions are ahown in Fig. 3. The proportional content of the α, β and γ structures is presented in Table 2. With the exception of the spectrum of rapeseed albumin fraction, the remaining spectra were characterized by similar shape. According to an interpretation acc. to Greenfield et al. [5] the profile of these spectra corresponds to the profile characteristic for proteins with a considerable content of the α and γ structures, and lower content of the β structure. The shape of spectrum CD of the rapeseed albumin fraction suggests a fairly large content of the γ structure. Since the albumin fraction of pareseed con-

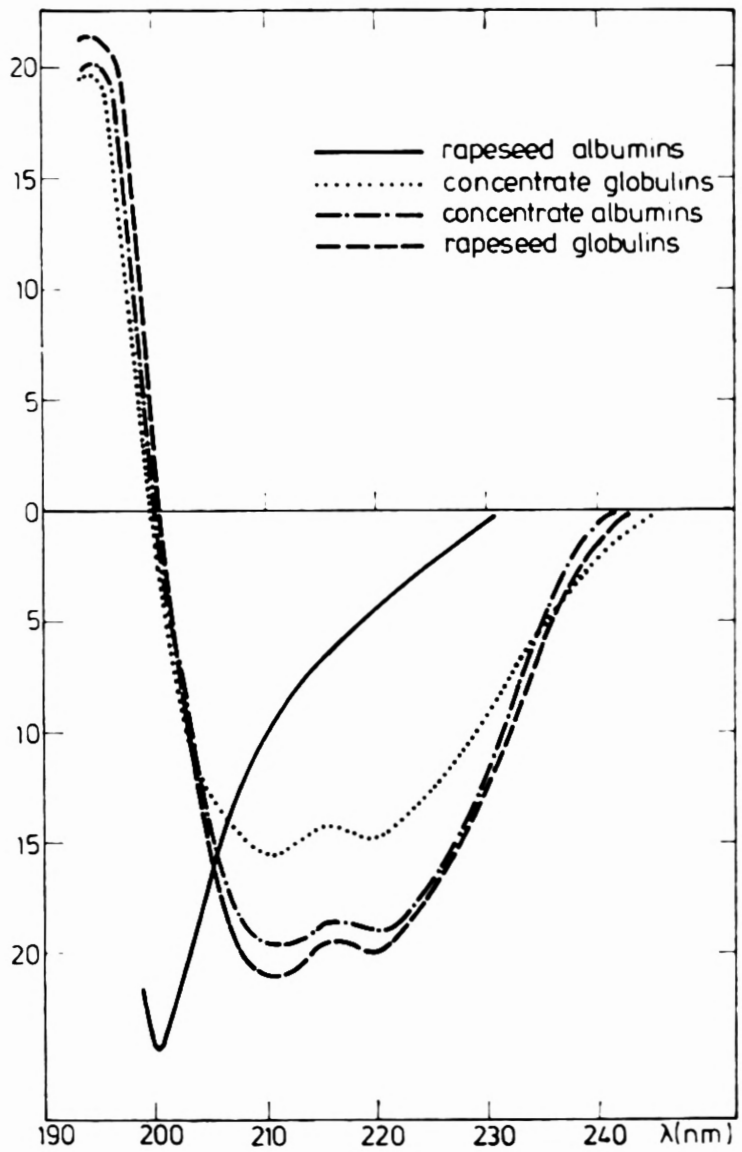

Fig. 3. CD spectra of rapeseed and concentrate proteins

Table 2. Proportional share of structures α, β and γ in the albumin and globulin fractions of rapeseed and concentrate, based on CD and IR spectra.

Protein type	Proportion of Structure			
	CD α	CD β	IR β	CD γ
Rapeseed albumin	—	—	18.0	—
Concentrate albumin	37.8	21.7	17.0	40.5
Rapeseed globulin	57.1	14.3	14.0	28.6
Concentrate globulin	50.2	12.1	14.0	37.6

stains phenolic acids which are also optically active, analysis of this spectrum is difficult [7].

The results allow for a determination of the range of changes taking place in the structure of proteins as a result of the process of concentrate production. In the albumin and globulin fractions of rapeseed and concentrate α and γ structures the have the highest proportional share in

conformation whereas the content of the β structure is about two to three times lower (Table 2). In the globulin fraction of the concentrate, the content of the α and γ structures decreased by 7% and 9% respectviely, and to a less degree (by about 2%) — the β structure. Although the proportional share of particular structures in the albumin fraction was not determined in the rapeseed, it can be assumed that the character of these changes is similar to those in the globulin fraction. On the basis of the infrared studies it was stated that the content of the structure in albumin fraction of rapeseed and concentrate was similar (17% and 18% respectively). It is highly probable that the content of the α and β structures decreased in the albumin fraction of rapeseed during the process of concentrate production, while the content of the gamma structure increased. These results are in accordance with the results obtained by Schwenke et al. [13].

CONCLUSIONS

1. In albumin and globulin fractions of rapeseed and concentrate, α and γ structures have the largest share in conformation whereas the content of the β structure was about 2-3 times lower.

2. The technological process of concentrate production caused a decrease in the content of the α and β structure by 7% and 9% respectively, and of the γ structure by about 2%.

3. The content of the α and β structures decreased in the albumin fraction of rapeseed during the process of concentrate production, while the content of the γ structure increased.

4. Albumin fractions form complexes with nucleic acids and (poly) phenols.

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

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S t r e s z c z e n i e

W pracy scharakteryzowano niektóre elementy konformacji białek frakcji albumin i globulin wyizolowanych z mączki i koncentratu białkowego uzyskanych z nasion rzepaku (patrz cz. I). Badania przeprowadzono przy wykorzystaniu spektrofotometrii w ultrafiolecie i podczerwieni oraz dichroizmu kołowego. W celu stwierdzenia obecności w frakcjach albumin i globulin koncentratu i nasion rzepaku kwasów nukleinowych poddano je hydrolizie kwasem mrówkowych, a następnie badaniom spektrofotometrycznym w UV. Szczególnie interesujące okazało się widmo frakcji albumin nasion rzepaku, które charakteryzowało się pasmem absorpcji przy 320 nm. Pojawienie się tego pasma można wytłumaczyć obecnością kwasów fenolowych związanych z tą frakcją. Zaobserwowany przy 260 i 280 nm wzrost absorbancji w przypadku frakcji globulin nasion rzepaku po ogrzewaniu z kwasem mrówkowym można wytłumaczyć obecnością związanych z tą frakcją kwasów nukleinowych. Analiza widm w podczerwieni frakcji albumin i globulin nasion rzepaku i koncentratu pozwoliła na wyznaczenie procentowej zawartości β struktury, która w obu frakcjach globulin wynosiła 14%, a w albuminach odpowiednio 18 i 17%. Analiza tych widm sugerowała również obecność w badanych frakcjach składników niebiałkowych, jak stwierdzone wcześniej kwasy fenolowe i nukleinowe. Wyniki badań tych samych frakcji za pomocą dichroizmu kołowego pozwoliły na określenie procentowych zawartości poszczególnych struktur α, β i γ. Wartości te wynosiły odpowiednio dla: albumin koncentratu 37,8, 21,7 i 40,7%, globulin nasion rzepaku 57,1, 14,3 i 28,6%, globulin koncentratu 50,2, 12,1 i 37,6%.