

STUDIES ON COMPREHENSIVE UTILIZATION FOR SUNFLOWER---II: ANALYSES OF SUNFLOWER SEED PROTEINS

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SUMMARY

The proteins extracted from the degreased sunflower seed dreg meal by the salt-extraction, alkali-extraction and salt-alkali-extraction methods were graded with Sephadex G-200 Gel Column in the study. The results showed that each of the three extractives contained three main constituents of the proteins. In the salt-extraction system, the constituent I was the lowest, consisting of 5-10% of the protein sample, the molecular weight was over 600,000, 280:260nm below 1; the constituent II was middle, consisting of 25-30% of the protein sample, the molecular weight was 350,000, 280:260nm below 1; the constituent III was the highest, consisting of 55-60% of the protein sample, the molecular weight was below 5,000, 280:260nm below 1. In the alkali-extraction system, the constituents II and III were the main compositions of the proteins. The molecular weights of the three constituents were different and all of 280:260nm were approximately 0.70. In the salt-alkali-extraction system, the three constituents were significantly different, the constituents II and III were the main compositions of the proteins and 280:260nm of the constituents I, II and III were 0.47, 0.70 and 0.50, respectively. The amino acid compositions in the degreased sunflower seed kernels, clear solution of the extractives and concentrates from the three extraction systems were also analyzed in the study. The results showed that the samples contained 16 common amino acids. Of the amino acids, Glx content was the highest and Met was the lowest. Of the necessary amino acids, Val had the highest content. Among the compositions, the ratio of the basic amino acids(Arg+Lys) to the acid amino acids(Glx+Asx) was below 0.5 or equal to 0.5 and the ratio of the necessary amino acids to the unnecessary amino acids was over 0.5.

INTRODUCTION

In recent years, sunflower has become a very important oil crop in many countries and its growing area and yield are on the increase. Sunflower oil has become the second important vegetable oil resource (1). Compared with other oil crops the dry degreased sunflower seed meal has higher protein content(2) and its necessary amino acid contents are higher than the cereal crops(3). Besides, the sunflower seed proteins do not contain any poisoning substances and easily processed for food or the concentrated protein food. For these 50 years the concentrated seed protein food has been widely used for human and animals since the functions, nutrition values and extracting processings of the proteins have continually learned. And sunflower proteins are the more significantly important vegetable protein resource and have been processed as a concentrated seed protein food(5

,6).

The sunflower protein can hardly dissolve in water, but easily in dilute salt solution. The globulin is the main part of the sunflower proteins. The biochemical characteristics and functions of this protein are different from the soybean proteins(2). Sabir et al.(1) graded the salt-dissolved sunflower proteins into five constituents with Sephadex G-200 Gel Column and studied the amino acid compositions of these constituents. Baudet et al.(3) graded the salt-dissolved proteins into three constituents: 1) about 20% of the light albumin; 2) 5-10% of the heavy albumin and 3) 70-80% of the globulin. The sunflower seed proteins obtained from the degreased sunflower seed dreg meal by the salt-extraction, alkali-extraction and salt-alkali-extraction were graded with Sephadex G-200 Gel Column and the amino acid compositions of all the constituents were analyzed in the study.

MATERIALS AND METHODS

The sunflower seed dreg (foot product from vegetable oil plants) was used as raw material. The dreg was mixed with n-butane in proportion of 1g of the dreg:10ml of n-butane to make suspension for degreasing the dreg. The suspension was refluxed for 1.5 hours and then filtered for recovering n-butane. Such prepared dry meal containing 45-50% of proteins was ready for the study.

The dry degreased sunflower seed dreg meal was tested by Kjeldahl's method. The proteins were extracted by the methods introduced in the part I of the paper -- "Extraction of Sunflower seed Proteins" and the proteins in the extractives were tested by the Ultra-violet Spectrophotometry and the method reported by Lowry et al.(7). After centrifuged in 14,000 rpm for 20 mins, the protein solution from the salt-extraction was directly injected into the column for grading the proteins or injected into it after dialyzed. The protein solution from the alkali-extraction or from the salt-alkali-extraction was injected into the column after dialyzed with the buffer solution (PH=8.0) of 0.2 molar phosphoric acid. OD280 and OD260 values of the proteins were tested with the Ultra-violet Spectrophotometer after eluted. For testing the molecular weights of the protein constituents: the V_0 of the gel column was first tested using blue glucan-2000 (molecular weight= 2×10^6); the V_e of the known protein---L-asparaginase was then tested and the molecular weight of the constituents was obtained by the regression equation(8). For analyzing amino acids: the extracted protein constituents and other samples were hydrolyzed with hydrochloric acid solution (6 equivalents) at 110 C for 24 hours. The hydrolyzed solution was tested by Model 835 Analyzer for Amino Acid (HITACHI, JAPAN).

RESULTS AND DISCUSSION

Gradation of the proteins and analyses of the amino acids in each of the protein constituents from the salt-extraction----

The undialyzed extractive solution of the degreased sunflower seed kernel and degreased sunflower seed dreg meal was respectively injected into Sephadex G-200 Gel Column after centrifuged. The results were shown in Fig. 1 and Fig. 2. Each of the two kinds of the extractive solution gave three peaks after graded. This showed that the degreased sunflower seed protein meal contained three salt-soluble constituents. The constituents I and II were much the same as the

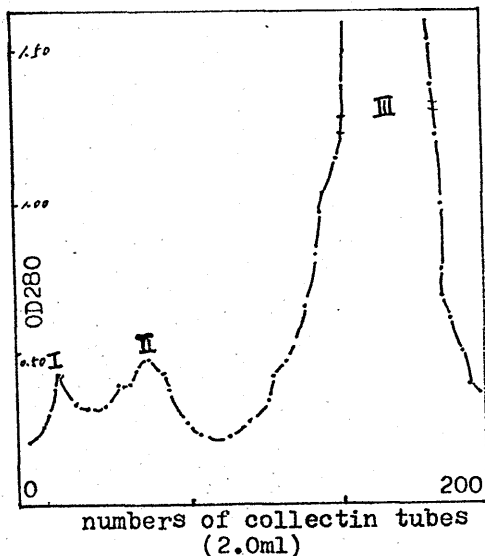


Fig. 1. Gradation of the undialyzed sunflower seed kernel proteins from the salt-extraction by Sephadex G-200 Gel Column.

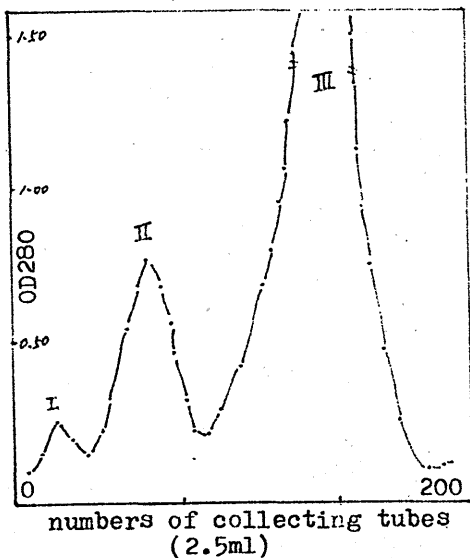


Fig. 2. Gradation of the undialyzed sunflower seed dreg proteins from the salt-extraction by Sephadex G-200 Gel Column.

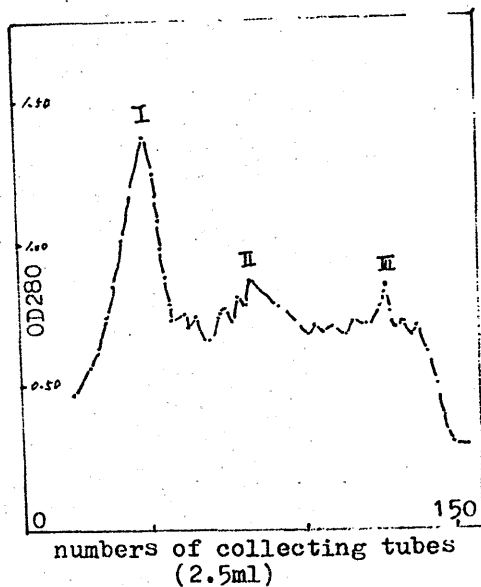


Fig. 3. Gradation of the sunflower seed proteins from the alkali-extraction by Sephadex G-200 Gel Column.

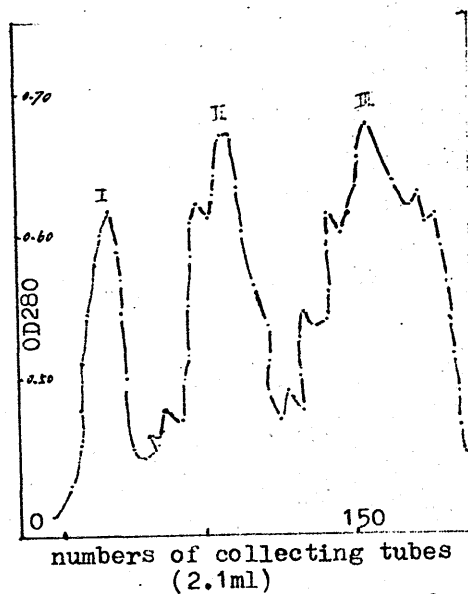


Fig. 4. Gradation of the sunflower seed proteins from the salt-alkali-extraction by Sephadex G-200 Gel Column.

constituents I and II reported by Sabir et al.(1), respectively. And these constituents could be firmly banded with chlorogenic acid by the covalent bonds or other bonds. This made the absorbing peaks of OD280 rise sharply(1,3). Of the three constituents graded by the column, the molecular weight of the constituent I was over 600,000 and it consisted of 5-10% of the protein sample; the molecular weight of the constituent II was about 350,000 and it consisted of 20-30% of the protein sample and the molecular weight of the constituent III was below 5,000 and it consisted of 55-60% of the protein sample. The results of the amino acid analyses of the three constituents were shown in Table 1. Except cystine and tryptophane, the seed kernel

Table 1. The amino acid compositions(% residue) in the sunflower seed kernel proteins and in the protein constituents from the salt-extraction.

Amino Acids	Seed kernel		Constituents		
	This study	Baudet data	I	II	III
Necessary AA					
Ile	43	45	54	52	38
Leu	64	65	65	60	54
Lys	34	36	33	19	45
Met	1	20	11	9	8
Phe	38	37	49	52	37
Thr	41	41	44	31	30
Val	62	63	82	81	75
Unnecessary AA					
Ala	63	65	68	60	69
Arg	73	68	42	56	41
Asx	91	93	92	95	92
Glx	214	196	180	199	178
Gly	100	102	133	95	124
His	20	22	12	18	11
Pro	81	49	168	123	161
Ser	59	55	55	43	37
Tyr	16	20	6	7	8

proteins and the constituents contained 16 common amino acids and the ratio of the necessary amino acids to the unnecessary amino acids was 0.39.

Gradation of the proteins and analyses of the amino acids in each of the protein constituents from the alkali-extraction----

The extractive solution was graded with Sephadex G-200 Gel Column and the results were shown as Fig. 3. The results showed that with the column grading the proteins, these protein constituents were also obtained from the soluble proteins extracted with alkali solution after the salt-extraction. The constituent I had the highest peak height and the constituent II and III had the smaller and the same peak heights. But each peak area of the constituents II and III was much more larger than that of the I. The indent curve line suggested that the molecular weights of the proteins were different. The ratio values of 280 to 260nm of the three constituents were 0.7. This suggested that they would be the complex of nucleoproteins.

The results of the amino acid analyses of the three constituents were shown in Table 2. Except cystine and tryptophane, each constituent contained 16 common amino acids.

Gradation of the proteins and analyses of the amino acids in each of the protein constituents from the salt-alkali-extraction----

Table 2. The amino acid compositions(% residue) in the sunflower seed kernel proteins and in the protein constituents from the alkali-extraction.

Amino Acids(AA)	Seed kernel	Constituents		
		I	II	III
Necessary AA				
Ile	43	53	56	46
Leu	64	76	72	64
Lys	34	31	25	56
Met	1	6	4	15
Phe	38	48	57	45
Thr	41	45	38	29
Val	63	76	77	73
Unnecessary AA				
Ala	63	70	67	51
Arg	73	61	63	59
Asx	91	100	110	90
Glx	214	148	183	155
Gly	100	127	97	128
His	20	17	18	14
Pro	81	77	68	111
Ser	59	60	60	66
Tyr	16	5	5	18

Table 3. The amino acid compositions(% residue) in the sunflower seed kernel proteins and in the protein constituents from the salt-alkali-extraction.

Amino acids(AA)	Seed kernel	Constituents		
		I	II	III
Necessary AA				
Ile	43	52	54	48
Leu	64	73	73	71
Lys	34	28	30	40
Met	1	16	9	33
Phe	38	44	60	35
Thr	41	47	42	38
Val	63	79	74	73
Unnecessary AA				
Ala	63	76	67	72
Arg	73	42	54	41
Asx	91	94	111	82
Glx	214	143	176	118
Gly	100	145	91	136
His	20	10	15	trace

The extractive solution was graded as mentioned above and the results were shown as Fig. 4. The soluble proteins from the salt-alkali-extraction still had three main peaks after graded under the same conditions.

The heights and areas of the three peaks were significantly different. The ratio values of 280:260nm of the three constituents were 0.47, 0.70 and 0.50, respectively. During the elution, there were some small peaks near the three main peaks. The results showed that the constituents II and III were the main protein compositions in the soluble protein solution from the salt-alkali-extraction and suggested that except the constituent I, other two had the different

molecular weights. It was expected that the three constituents contained the same constituents from the salt-extraction or from the alkali-extraction. During the gradation, there was no chlorogenic acid to be found.

The results of the amino analyses of the three constituents were shown in Table 3. The ratio values of the necessary amino acids to the unnecessary amino acids of the constituents I, II and III were 0.51, 0.52 and 0.51 and the ratio values of the basic amino acids to the acid amino acids were 0.30, 0.29 and 0.41, respectively.

The amino acid compositions in the sunflower seed protein concentrates from the three extracting methods----

In the study, the proteins from the three extracting systems were prepared into their concentrates. Then, the concentrates were hydrolyzed for analyzing the amino acid compositions. The results were shown in Table 4 and showed that the protein concentrates from each

Table 4. The amino acid compositions(% residue) in the sunflower seed protein concentrates from different extracting methods.

Amino acids(AA)	Seed kernel	Concentrates		
		Salt-extraction	Alkali-extraction	Salt-alkali-extraction
Necessary AA				
Ile	43	54	52	56
Leu	64	69	74	71
Lys	34	23	36	28
Met	1	1	trace	4
Phe	38	52	45	51
Thr	41	35	43	35
Val	63	77	86	77
Unnecessary AA				
Ala	63	66	78	62
Arg	73	83	58	85
Asx	91	123	89	118
Glx	214	245	169	249
Gly	100	89	95	89
His	20	25	21	25
Pro	81	trace	102	trace
Ser	59	42	38	40
Tyr	16	14	15	15

each of the three extraction methods contained 16 amino acids. The prepared sunflower seed concentrates had higher nutrition values. The ratio values of the necessary amino acids to the unnecessary amino acids in the three protein concentrates from the salt-, alkali-extractions were 0.50, 0.51 and 0.47, respectively and those of the basic amino acids(Arg+Lys) to the acid amino acids(Glx+Asx) were 0.29, 0.36 and 0.31, respectively.

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