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Differences in the Chemical Composition of Some Pea Proteins

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Vicilin and legumin, the two globulins in seeds of the pea, *Pisum sativum*, were shown by Osborne and Campbell [1] to have different elementary composition. Thus especially the sulfur content of legumin is much higher than that of vicilin. The same differences were observed if the two globulins had been prepared from seeds of other plants belonging to the family *Leguminosae*. It is, however, not probable that the proteins analyzed by Osborne were homogeneous because his method of separation of the two globulins [fractionation by precipitation with $(NH_4)_2SO_4$] give protein preparations which are inhomogeneous by ultracentrifugation [2].

Earlier investigations published from this Institute [2,3] have described fractionations of vicilin and legumin by which the two globulins are obtained homogeneous as tested by both ultracentrifugation and electrophoresis. In this paper some determinations on the amino acid composition of the pea proteins are reported. Determinations have been carried out for only a few amino acids, which could be determined by simple methods, because preliminary experiments using paper chromatography showed that the differences in the amino acid contents between the pea proteins were not very large. The main problem was to settle whether or not any differences in the amino acid composition of the different pea proteins could be observed.

A. Preparation of the pea proteins

The homogeneous preparations of vicilin and legumin were prepared according to a method described in a previous paper [3]. The albumin preparation used in the present investigation was prepared according to the following method.

Ground ripe pea seeds (field variety "Torsdagsärt II") are extracted over night at + 4° C with 0.2 *M* NaCl, pH 7. The extract is centrifuged and filtered clear and then

dialysed in a cellophan bag against water (24 hours against running tap water, then distilled water). By this procedure the globulins are precipitated when the salt concentration is lowered, and low-molecular nitrogenous compounds (amino acids, peptides *etc.*) pass through the bag. After centrifugation the supernate is dialysed once more, until no precipitate is obtained by prolonged dialysis. When all globulins have been removed the supernate contains only albumins. The solution is cooled to − 16° C and dried *in vacuo* over $CaSO_4$. In this way the albumin fraction is obtained in the form of a white powder which is easily soluble in water. It is, however, not homogeneous in the ultracentrifuge and in the electrophoresis apparatus.

As can be seen from Fig. 1 d two inhomogeneous components are obtained in the ultracentrifuge diagrams [4] after about 90 minutes at 300 000 g. Below, this albumin fraction will be called "albumin". Fig. 1 a shows an ultracentrifuge diagram of an extract obtained from peas with 0.2 *M* NaCl, pH 7. Three peaks can be seen in the diagram, corresponding to, from the left, 1) albumins + low-molecular material, 2) vicilin and 3) legumin. A similar diagram is obtained even after a few minutes' extraction. Thus, vicilin and legumin are extracted from the seed material in the form of two different protein components, and are not products formed during the purification process. Fig. 1 b shows the composition of the solution after 48 hours' dialysis of the extract against the extraction buffer by which procedure the low-molecular material has been removed, and the solution contains albumin, vicilin and legumin. Fig. 1 c shows the composition of the precipitate which is obtained when the solution containing albumin, vicilin and legumin is dialysed against water. Only the two globulins are precipitated, and the albumin is left in the supernate (Fig. 1 d). If the precipitate containing vicilin and legumin is treated with a solution of 0.2 *M* NaCl, pH 4.7, the vicilin dissolves but legumin does not. After centrifugation, traces of legumin are removed from the supernate by dialysis against a buffer of pH 4.7 and vicilin is obtained free from legumin (Fig. 1 e). The precipitate from the centrifugation, which contains legumin contaminated with small amounts of vicilin, is dissolved in 0.2 *M* NaCl, pH 9.2 and the solution dialysed against 0.2 *M* NaCl, pH 4.7. Legumin precipitates, and by repeated dialysis at pH 4.7 it is completely separated from vicilin, as can be seen in Fig. 1 f.

B. Nitrogen content of the pea proteins

Nitrogen determinations according to Kjeldahl were carried out on the freeze-dried protein preparations. The determinations were carried out on samples containing 0.5—1 mg N with an experimental error of ± 1 %. The values in Table 1 are average values from four determinations. The water content, about 7 %, was determined by heating 0.3—0.5 g of protein to 105° C for 24 hours. The values in Table 1 are corrected for water content but not for ash.

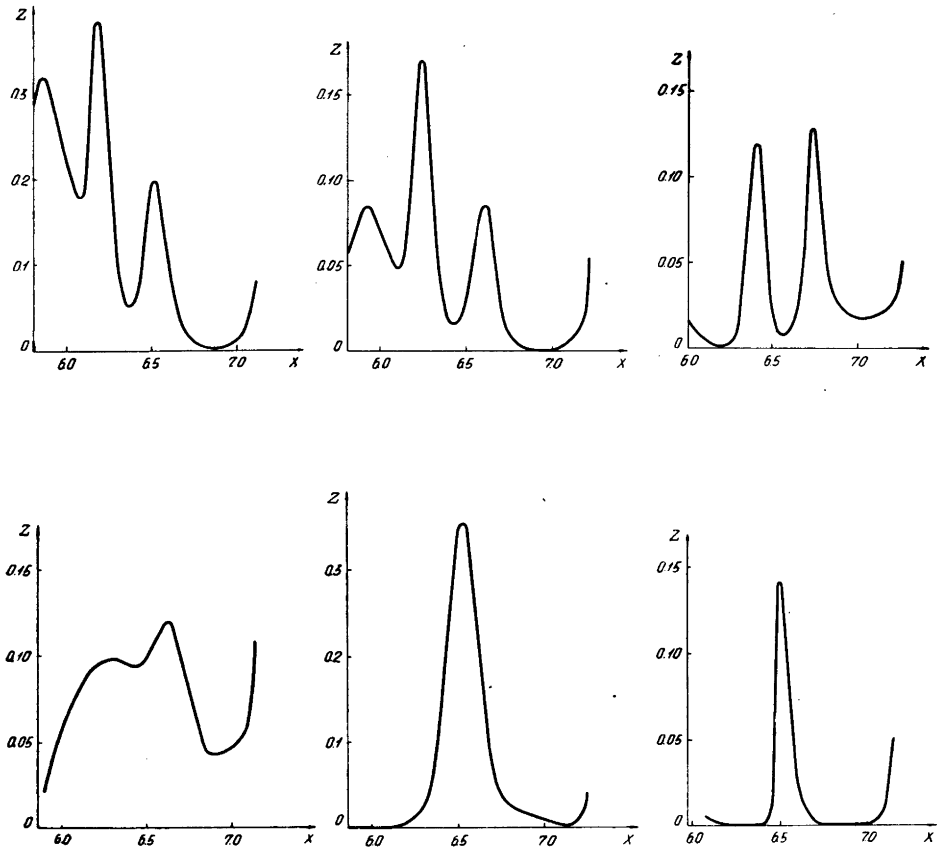

Fig. 1. Ultracentrifuge diagrams of pea proteins at different stages of the purification process. The abscissae represent distance from the axis of rotation (cm.) and the ordinates represent the scale-line displacement (mm.); t = time in minutes after reaching full speed and b = distance between cell and scale in mm.

a) *Top left. Original extract. t = 35′, b = 60 at 60.000 r.p.m.*
b) *Top centre. Original extract after dialysis against 0.2 M NaCl, pH 7. t = 35′, b = 60 at 60.000 r.p.m.*
c) *Top right. Original extract, dialysed against H_2O. The precipitate after dissolving in 0.2 M NaCl, pH 7. t = 25′, b = 60 at 65.000 r.p.m.*
d) *Bottom left. Original extract, dialysed against H_2O. The supernate after concentration (albumin). t = 140′, b = 60 at 60 000 r.p.m.*
e) *Bottom centre. Final preparation of vicilin. t = 160′, b = 40 at 40.000 r.p.m.*
f) *Bottom right. Final preparation of legumin. t = 15′, b = 20 at 65.000 r.p.m.*

Table 1. Nitrogen content of pea proteins.

Preparation	% N	% N Average values
Vicilin no. 10	17.32	
» no. 22	17.60	17.5
» no. 25	17.60	
Legumin no. 8	18.50	
» no. 11	17.35	17.9
» no. 24	17.86	
Albumin no. 1	15.07	15.1

As can be seen from Table 1 the nitrogen content is not the same in the different preparations of the same protein. Osborne and Campbell [1] determined the nitrogen content of vicilin to 17.40 % and of legumin to 18.04 %. Thus, the agreement between their values and the values in Table 1 can be considered satisfactory. The nitrogen content of the albumin used in these investigations is very low, but determinations on other albumin preparations prepared in the same way have shown that the value in Table 1 is correct.

C. Determination of tyrosine and tryptophan

In a previous paper it was preliminary shown that legumin contains more tryptophan than vicilin [5]. The method used for the determination of tyrosine and tryptophan is based on measurement of light absorption in the ultra violet region of a protein solution in 0.1 *N* NaOH [6-8]. Only the aromatic amino acids and cystine have any measurable absorption in the region 250—320 mμ [4]. By selecting the wave-length region 280—320 mμ the absorption of phenylalanine can be neglected, and the absorption measured is that of tyrosine, tryptophan and cystine. The absorption of cystine can not be neglected at 280 mμ when the amounts of tyrosine and tryptophan are calculated, unless the cystine content is very low compared with the amounts of tyrosine and tryptophan. Preliminary determinations with the phosphotungstic acid method according to Schöberl and Rambacher [10] of the cyst(e)ine (cysteine + cystine) content gave the value 0.25 % cyst(e)ine N/total N for vicilin and 0.42 % for legumin. Thus, the cyst(e)ine contribution to the absorption can be neglected.

Experimental: 25 mg dried protein are dissolved in 7.5 ml 0.1 *N* NaOH and the solution is filtered. Two nitrogen determinations are carried out on 1.0 ml each. The solution is then diluted 4 times (in case of albumin 7 times) with 0.1 *N* NaOH and the extinction is measured in a Beckman spectrophotometer with 0.1 *N* NaOH as reference solution. For the calculation of the tyrosine and tryptophan contents the extinction is usually measured at 280, 294.4, 340 and 370 mμ [8,5] but in these experiments the whole absorption curve was measured in the region 250—370 mμ. For the calculation of the tyrosine and tryptophan content see Goodwin and Morton [10] and Danielsson [8]. The results of the measurements are found in Table 2 and Fig. 2.

As can be seen from Table 2, the tyrosine N in % of total N is determined with a maximal deviation from the average of ± 5 %. The corresponding value for tryptophan is ± 15 %.

Table 2. Determinations of the tyrosine and tryptophan contents in pea proteins by measurement of light absorption in the ultra violet region. The extinction values (E) are observed values.

Preparation	Concentration mg N/ml	E_{280}	$E_{294.4}$	E_{340}	E_{370}	Tyrosine N in % of total N	Tryptophan N in % of total N
Vicilin no. 10	0.127	0.389	0.480	0.020	0.003	1.87	0.28
	0.135	0.410	0.500	0.035	0.024	1.79	0.33
Vicilin no. 22	0.132	0.390	0.485	0.020	0.010	1.86	0.27
	0.147	0.445	0.555	0.021	0.010	1.93	0.31
Vicilin no. 25	0.139	0.420	0.518	0.039	0.029	1.82	0.28
	0.151	0.466	0.572	0.029	0.018	1.88	0.33
						Av.: 1.86	0.30
Legumin no. 8	0.132	0.580	0.560	0.035	0.020	1.75	0.98
	0.133	0.690	0.650	0.082	0.060	1.85	1.03
Legumin no. 11	0.140	0.678	0.650	0.050	0.028	1.82	1.14
	0.146	0.705	0.680	0.070	0.048	1.78	0.90
Legumin no. 24	0.131	0.540	0.545	0.028	0.015	1.77	0.92
	0.146	0.680	0.688	0.075	0.056	1.89	0.94
						Av.: 1.81	0.99
Albumin no. 1	0.078	0.628	0.590	0.046	0.031	2.92	2.05
	0.068	0.534	0.490	0.040	0.023	2.77	2.10
	0.074	0.610	0.560	0.051	0.031	2.77	2.07
						Av.: 2.82	2.07

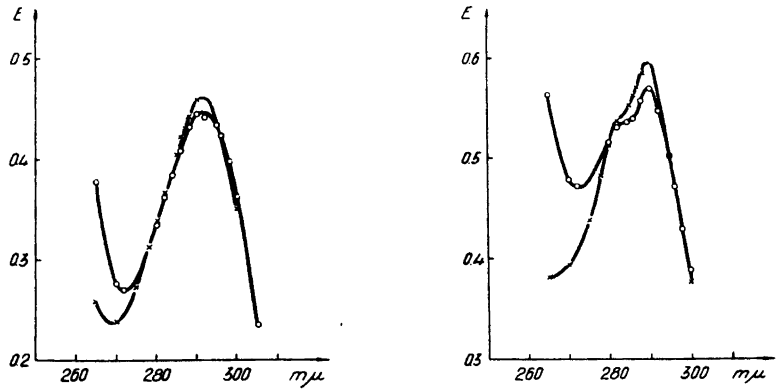

Fig. 2. Experimental (○) *absorption curves in the ultra violet region for vicilin (left) and legumin (right) in 0.1 N NaOH.* (×) *represent theoretical curves obtained by adding the extinctions of tyrosine and tryptophan in the concentrations calculated from the experimental curves.*

Fig. 2 shows the agreement between the experimental curves and the theoretical curves, obtained by adding the extinction of pure tyrosine and tryptophan in the same concentration as was calculated from the experimental curve. The agreement in the wave length region used for the calculation of the tyrosine and tryptophan contents must be considered satisfactory.

D. Determination of acid and basic amino acids

The acid and basic amino acids were separated by electrodialysis according to Theorell and Åkesson[11] with the modifications indicated by Weibull[12].

Experimental. 40 mg of protein were hydrolyzed for 30 hours with 19 % HCl on a boiling water bath under a reflux condenser. After addition of diluted H_2SO_4 in equivalent amount to the total nitrogen present the hydrolysate was evaporated three times to dryness in order to remove HCl, because chlorine may destroy the acid amino acids during the electrodialysis[11]. The amino acid mixture was then dissolved in 25 ml water. No humin formation was observed. During the electrodialysis the ammonia formed in the cathode compartment was taken up in 25 ml 0.1 *N* HCl. The amide nitrogen was determined by distillation according to Theorell and Åkesson[11]. The basic amino acids were determined according to Macpherson[13]. The acid amino acids were determined by separation on an ion exchange column (Dowex 50) combined with automatic, continuous measurement of the electrolytic conductivity of the eluate. By this method the ratio between the concentrations of glutamic acid and aspartic acid is obtained. The method has been worked out by Drake[14] at this Institute.

Table 3. The distribution of acid, neutral and basic amino acids in pea proteins from electrodialysis experiments, and quantitative determinations of some separate amino acids. All values are expressed as specific N in % of total N.

Preparation	Acid amino acids	Neutral amino acids	Basic amino acids	Amide nitrogen	Recovery %	Arginine	Histidine	Lysine	Glutamic acid	Aspartic acid
Vicilin no. 10	21.64	39.47	29.24	10.00	100.35	21.00	3.59	4.65	12.24	9.40
» no. 22	20.21	40.96	30.90	9.96	102.03	20.87	3.71	6.32	11.73	8.48
» no. 25	22.49	38.46	29.54	10.64	101.13	21.62	3.75	4.17	12.48	10.00
Av.:	21.45	39.63	29.89	10.20		21.16	3.68	5.05	12.15	9.29
Legumin no. 8	25.03	34.85	32.46	10.44	102.78	23.42	4.54	4.50	15.95	9.09
» no. 11	24.98	33.96	31.30	10.81	101.05	23.63	4.55	3.12	15.82	9.16
» no. 24	25.70	34.02	31.94	10.57	102.23	23.68	4.60	3.66	15.61	10.09
Av.:	25.24	34.28	31.90	10.61		23.56	4.58	3.76	15.79	9.45
Albumin no. 1	20.23	40.50	29.62	8.58	98.93	10.73	5.59	13.30	10.61	9.61
	20.00	40.84	29.43	8.91	99.18	10.87	5.82	12.74	10.02	9.98
	20.18	40.05	30.37	8.14	98.74	11.14	6.00	13.23	9.88	10.30
Av.:	20.14	40.46	29.81	8.54		10.91	5.80	13.09	10.17	9.96

The results of the measurements are given in Table 3. In the separation of acid, neutral and basic amino acids, the greatest error was obtained when vicilin was analyzed, with a maximal deviation from the average of $\pm$ 6 %. The corresponding value for legumin and albumin is $\pm$ 2 %. It is possible that the different vicilin preparations were not completely homogeneous and this fact caused the greater part of the error in these determinations. A more probable explanation, however, is that the first determinations were carried out on vicilin and the experimental accuracy was higher when legumin and albumin were analyzed. According to Weibull [12] 1.5 % of the acid amino acids remain in the mixture of neutral amino acids in the method used. The values in Table 3 are not corrected for this error, but in Table 4 the values are corrected. The values in Table 3 for amide nitrogen are a little too high depending on the decomposition of serine and threonine [12]. In the determinations of the basic amino acids the maximum deviation from the average between the different preparations is $\pm$ 2 % for arginine, $\pm$ 4 % for histidine and $\pm$ 25 % for lysine. The corresponding values for the acid amino acids are $\pm$ 4 % for glutamic acid and $\pm$ 9 % for aspartic acid. The great error in the determination of lysine probably depends on the fact that lysine is deter-

Table 4. Amino acid content (% of weight) of some seed proteins.

Amino acid	Vicilin	Legumin	Albumin	Edestin[17]	Arachin[18]	Glycinin[18]
Tryptophan	0.4	1.3	2.3	1.48	0.6	0.4
Tyrosine	4.2	4.2	5.5	4.34	4.5	2.7
Arginine	11.5	13.1	5.1	16.7	15.4	11.7
Histidine	2.4	3.0	3.2	2.9	1.9	0
Lysine	4.6	3.5	10.3	2.4	2—4	6
Glutamic acid	22.7	30.1	16.3	20.7	17	19
Aspartic »	15.7	16.3	14.5	12.0	5	2

mined by difference. Thus, when the lysine content is low, the accuracy of the method is comparatively low.

E. A comparison of the compositions of the pea proteins

As to the distribution of acid, neutral and basic amino acids in the pea proteins, it can be seen that vicilin and albumin are very similar. It is evident that legumin has a higher content of acid and basic and a smaller content of neutral amino acids than vicilin. These differences are considerably greater than the experimental errors. Legumin has the same amount of amide nitrogen as vicilin. Albumin contains less amide nitrogen.

Some conclusions concerning the composition of the pea proteins can be drawn if the errors in the determinations of the separate amino acids are taken into consideration. According to Table 4 legumin contains 3 times and albumin 6 times more tryptophan than vicilin does. The tyrosine content is the same in vicilin and in legumin but higher in albumin. The arginine content is considerably higher in legumin than in vicilin but remarkably low in albumin. Legumin and albumin have a higher content of histidine than vicilin. As there are big errors in the determinations of the lysine content, it is impossible to say if vicilin and legumin contain different amounts of this amino acid. The lysine content of albumin, however, is very high and as far as the author knows higher than for any other seed protein investigated. Legumin has more and albumin less glutamic acid than vicilin. The aspartic acid content is the same for the three proteins.

DISCUSSION

A comparison of the amino acid compositions of vicilin and legumin shows that clear differences exist in the tryptophan, arginine, histidine and glutamic acid content of the two proteins. Differences can also be seen in the distribution of acid, neutral and basic amino acids. These results together with those obtained earlier from ultracentrifuge and electrophoresis experiments show clearly that vicilin and legumin are two well-defined and different globulins.

The albumin fraction is quite different from the two globulins both in chemical composition and physical-chemical properties. Characteristic of the albumin fraction is its high tryptophan and lysine contents and low arginine content. The clear character of reserve proteins of the globulins was shown by germination experiments on pea seeds [15]. The albumin fraction probably has a very small importance as a reserve protein, but it contains many enzymes. A proteolytic enzyme has been isolated from this fraction [16], and the albumin fraction also contains a peroxidase and a dipeptidase.

The results of the measurements are found in Table 4, where the compositions of the pea proteins are compared with those of three other important seed proteins [17,18]. Compared with these seed proteins the pea globulins are characterized by their high content of acid amino acids. The high content of tryptophan and lysine in the albumin fraction is very remarkable and is reminiscent of the values which have been found for animal proteins, *e.g.* β-lactoglobulin. Lysine, being one of the essential amino acids for the chicken, and probably for man, is often present in too low a concentration in plant proteins, especially in the cereal proteins, to give these proteins a sufficiently high nutritive value [14]. Table 4 shows that the pea proteins, especially the albumin fraction, can be important from a nutritive point of view.

SUMMARY

1. Quantitative determinations of tyrosine, tryptophane, arginine, histidine, lysine, aspartic acid and glutamic acid in vicilin, legumin and albumin from pea seeds are reported.

2. Vicilin and legumin have different amino acid composition and are two well-defined and different globulins.

3. The amino acid composition of the albumin fraction is quite different from that of the globulins. No relationship has been observed between the globulins and the albumins.

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