COMPARATIVE STUDY OF THE PROTEINS OF DIFFERENT CORN CULTIVARS USING FRACTIONATION BY HPLC GEL CHROMATOGRAPHY

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Received May 5, 1985

Summary

Proteins in samples of nine cultivars grown in Hungary and other countries were investigated. On the basis of fractionation by HPLC it was proved that corn samples 7 and 9 (grown in USA and Egypt) give for all the protein fractions similar results, while sample 8 (grown in FRG) is more similar to cultivars grown in Hungary (Table 1)

The protein distribution of cultivars grown in Hungary shows characteristic differences, and on the basis of the distribution of the different fractions different similarities.

Introduction

While wheat proteins were extensively studied since a long time and several very important problems were cleared (baking quality of protein, nutritional value, effect of fertilization on the proteins, variety identification on the basis of the protein spectrum etc.), this cannot be said of corn proteins. This is true for both Hungary and Egypt, research results published concern a relatively narrow field, and more intensive research started only in recent years. Research in this direction was first motivated by demands arising in conjunction with modern feeding and with the improvement of the efficiency of meat production. The key issue was the increasing of the low tryptophan and lysine content of corn proteins. Later further and further problems had to be solved. Thus the technological quality of corn in conjunction with its industrial utilization (starch manufacture, brewery, food products of the milling industry, extruded products, sweet corn, corn germ etc.), and the elucidation of the relationships between the proteins became the questions of the day. Eventually the complex system of corn qualification has to be developed on the basis of research results [1].

Cereal proteins can be separated by several methods. Following fractionation with solvents, conventional gel chromatography was most often used. Gel electrophoresis proved to be for a long time the most efficient method.

High performance liquid chromatography is one of the youngest members of the family of chromatographic methods. Its application in food sciences looks back on a past of about 10 years [2].

While initially it was used only for the investigation of components of low molecular weight, later it became suitable, primarily by the qualitative development of column packings, for the investigation of macromolecules, and thus of proteins. Investigation of cereal proteins by high performance liquid chromatography dates back to but a few years [3]. A still more recent development is reverse phase high performance liquid chromatography [4]. The importance of these techniques is that they make possible a more efficient and quicker separation, than methods used so far.

Experiments

Materials and methods

Corn varieties tested are given in Table 1.

Table 1

List of the investigated cultivars

Number	Cultivar	Country
1	MV-SC 394 (flint)	Hungary
2	Pioneer 3732 SC (dent)	Hungary
3	MV-SC 550 (waxy)	Hungary
4	SC-6390 HL (opaque-2)	Hungary
5	White sweet corn	Hungary
6	Sweet corn MV-SC-Ideal	Hungary
7	White flint	USA
8	Brillant (dent)	FRG
9	Giza-2 (dent)	Egypt

Extraction of proteins

Albumins

0.5 g of defatted corn meal was weighed into a plastics centrifuge tube, and 3 cm^3 distilled water was added. After shaking for 1 hour, the suspension was centrifuged for 20 minutes at a speed of 5000 r.p.m., then the supernatant was decanted. The residue was extracted for a second time in a similar way. The combined supernatants were used for further investigation.

Globulins

The water-insoluble substance was extracted with 3 cm^3 of 1 mol/dm^3 NaCl solution in the same way as described for albumin extraction.

Glutelin and zein

12 g pulverized corn meal was mixed in a conical flask with 360 cm^3 potassium hydroxide solution containing alcohol (0.05 mol/dm^3 KOH solution containing 60% ethanol). After shaking for 1 hour the suspension was centrifuged for 30 minutes at a speed of 5000 r.p.m. The supernatant liquid was decanted and adjusted with concentrated hydrochloric acid to pH = 6.5. It was kept standing for 24 hours in a refrigerator for the complete precipitation of glutelin. The precipitate was separated by centrifuging, and dried at room temperature. The supernatant liquid was diluted with distilled water to twofold, then kept standing for 48 hours in a refrigerator for an as far as possible complete precipitation of zein. The precipitate was separated by centrifuging and dried at room temperature.

Chromatographic separation

For the high performance liquid chromatographic tests 20 mg protein was dissolved in 0.2 M NaH_2PO_4 buffer containing SDS (0.2%), of pH 4.15. A Waters' type chromatograph was used. Column packing was TSK 4000

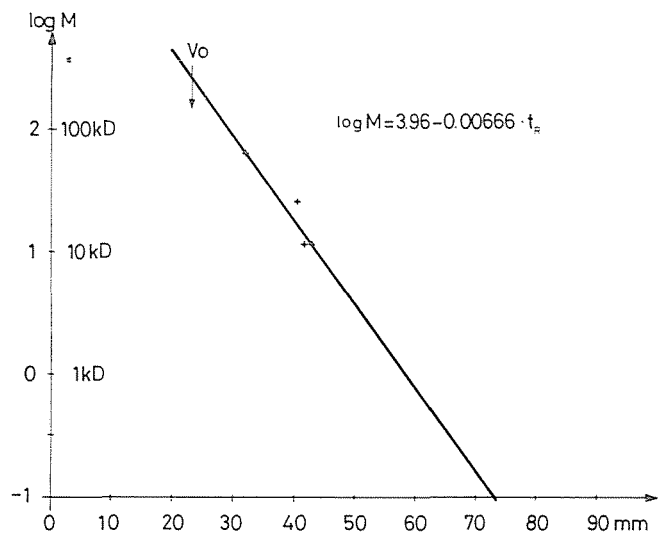

Fig. 1. Calibration curve for the determination of molecular weight

Table 2

Molecular mass distribution of albumins and relative quantities of fractions (% of total albumin)

Country	Cultivar	Molecular mass (kD)									
		Fraction I 280	II 200	III 25	IV 22	V 12	VI 4.5	VII 3.5	VIII 2.5	IX 1.8	X 0.8
Hungary	MV-SC 394 (flint)	—	10.02	—	12.44	9.73	3.00	—	28.40	26.37	10.42
	Pioneer 3732 SC (dent)	7.65	13.17	—	11.98	13.19	3.53	—	29.42	21.02	—
	MV-SC 550 (waxy)	—	18.95	—	14.27	13.87	3.43	—	25.21	24.26	—
	SC 6390 HL (opaque-2)	—	9.18	—	30.26	—	—	—	60.31	—	—
	White sweet corn	—	—	7.28	10.61	—	1.11	—	38.48	42.50	—
	Sweet corn MV-SC-Ideal	—	—	7.29	11.14	—	1.33	—	39.02	41.18	—
FRG	Brillant	—	19.40	11.69	15.62	—	3.10	26.93	23.24	—	—
USA	White flint	5.55	7.01	—	—	62.71	—	—	—	—	24.71
Egypt	Giza-2	—	10.11	—	37.19	—	—	52.68	—	—	—

Table 3

Molecular mass distribution of globulins and relative quantities of fractions (% of total globulin)

Country	Cultivar	Molecular mass (kD)								
		Fraction I 200	II 50	III 22	IV 14	V 7.5	VI 3.4	VII 2	VIII 1.8	IX 1.3
Hungary	MV-SC 394 (flint)	8.90	3.89	17.24	11.49	7.31	30.21	—	14.57	6.37
	Pioneer 3732 SC (dent)	4.61	4.29	25.17	7.09	5.19	25.37	11.43	13.62	3.24
	MV-SC 550 (waxy)	4.88	4.64	20.76	8.02	5.41	17.63	18.46	17.23	2.97
	SC 6390 HL (opaque-2)	3.98	4.79	15.51	7.86	4.90	20.45	19.76	17.37	5.30
	White sweet corn	—	4.53	22.68	7.28	—	37.94	27.51	—	—
	Sweet corn MV-SC-Ideal	—	4.39	18.48	15.50	—	61.61	—	—	—
FRG	Brillant	9.24	2.94	34.03	8.74	—	45.05	—	—	—
USA	White flint	5.05	5.64	35.55	12.14	—	41.62	—	—	—
Egypt	Giza-2	7.84	4.47	20.87	13.79	7.45	45.57	—	—	—

sW/300, flow rate 1 cm^3/min, pressure 70 bar. An UV detector was used at 280 nm and a 4-channel compensograph. The chromatograms were quantitatively evaluated on the basis of the peak areas with an integrator Digint 34 μ, manufactured by Chinoin (Hungary). The quantitative ratios of the single fractions were calculated with an integrator. For the calibration of the column a mixture of proteins of known molar mass was used, and the retention time of the protein was plotted as a function the logarithm of the molar mass. In the case of the separation column used, points lie along a straight line in the molar mass range 10—200 kD (see Fig. 1), which was determined by the least square method. The equation is the following:

$$\log M = 3.96 - 0.006\, t_R ,$$

where t_R is the retention time in sec, M is the molar mass, kD, and with the aid of the molar mass it could be estimated within an accuracy of $4 \pm$ kD.

Peaks observed in the range of molar masses below 10 000 are polypeptides, or were attributed to compounds with absorption in the UV range, eluted together with proteins.

Results and discussion

Tables 2—5 give the protein quantities falling into the single range. The headpiece of the tables contains the molar masses to be assigned to the single peaks. Figures 2—17 show characteristic types of chromatograms.

Table 2 contains the test results of the albumin fraction obtained by HPLC.

Substantial and characteristic differences can be observed between the albumin fractions with respect to protein distribution.

Table 4

Molecular mass distribution of zeins and relative quantities of fractions (% of total zein)

Country	Cultivar	Molecular mass (kD)					
		Fraction I 200	II 105	III 45	IV 18	V 2.5	VI 2
Hungary	MV-SC 394 (flint)	4.05	17.32	51.24	27.38	—	—
	Pioneer 3732 SC (dent)	9.88	20.21	34.19	35.69	—	—
	MV-SC 550 (waxy)	11.24	21.49	37.85	29.43	—	—
	SC 6390 HL (opaque-2)	8.38	14.33	42.34	34.94	—	—
	White sweet corn	5.83	9.94	31.03	40.32	6.53	6.35
	Sweet corn MV-SC-Ideal	9.30	18.18	44.97	27.50	—	—
FRG	Brillant	3.28	14.19	47.30	35.23	—	—
USA	White flint	18.88	20.15	35.25	25.67	—	—
Egypt	Giza-2	4.19	15.62	47.52	32.63	—	—

Table 5

Molecular mass distribution of glutelins and relative quantities of fractions (% of total glutelin)

Country	Cultivar	Molecular mass (kD)						
		Fraction I 200	II 80	III 45	IV 18	V 3	VI 2.5	VII 2
Hungary	MV-SC 394 (flint)	12.13	18.14	27.22	21.87	14.30	—	6.22
	Pioneer 3732 SC (dent)	25.41	10.27	14.78	38.00	5.82	—	5.64
	MV-SC 550 (waxy)	20.95	26.26	33.56	15.07	1.31	1.44	1.41
	SC 6390 HL (opaque-2)	16.54	21.26	28.55	20.59	8.80	—	4.24
	White sweet corn	18.75	21.88	30.97	18.00	7.24	—	3.12
	Sweet corn MV-SC Ideal	19.57	13.25	19.99	32.99	8.71	—	5.46
FRG	Brillant	7.08	—	25.19	22.76	44.89	—	—
USA	White flint	25.27	15.86	23.53	17.66	14.53	—	3.09
Egypt	Giza-2	7.94	—	28.41	23.88	39.74	—	—

The major part of UV absorbance (near 80%) falls within the range below 25 kD, 7—20% arises from protein falling into the exclusion range. The substantial part of UV absorption falls into the range below 10 kD. In the case of samples 5 and 6 this is 82%, in that of sample 8 only 25%, while in the case of other samples 53—54%, and in that of sample 1 68%. This can be partly attributed to degraded proteins or to UV-active compounds dissolved together with proteins. These bands can be observed also in the case of globulin fractions, their quantity varying between 42 and 62%. 4—10% of the globulin fractions falls into the range of molar mass extracted, while the rest between 10 and 50 kD. While in the case of albumin fractions the absence of fractions falling into certain molar mass ranges can be readily observed, with globulins the differences are rather of quantitative character.

In the distribution of zein fractions, UV-absorbing component below 10 kD was observed only in a single case, and presumably this got as impurity into the sample.

In the zein samples four principal fractions were observed, separated in the form of rather sharp peaks from one another; 18, 45 and 105 kD. The similarity of corn samples was investigated by cluster analysis. Considering the quantity of protein fractions obtained by HPCL as coordinate, similarity was evaluated by the similarity criterion based on the Euclidean distance. To eliminate correlation between the single fractions, principal component analysis was performed, and similarity was calculated using the principal components. The location of the single corn samples in the plain of the first two principal components was also investigated, as this contained a considerable part of the variance of the total system (see Figs 18—29) (in the case of albumin 58, of globulin 68.5, of zein 89 and in that of glutelin 83.2%).

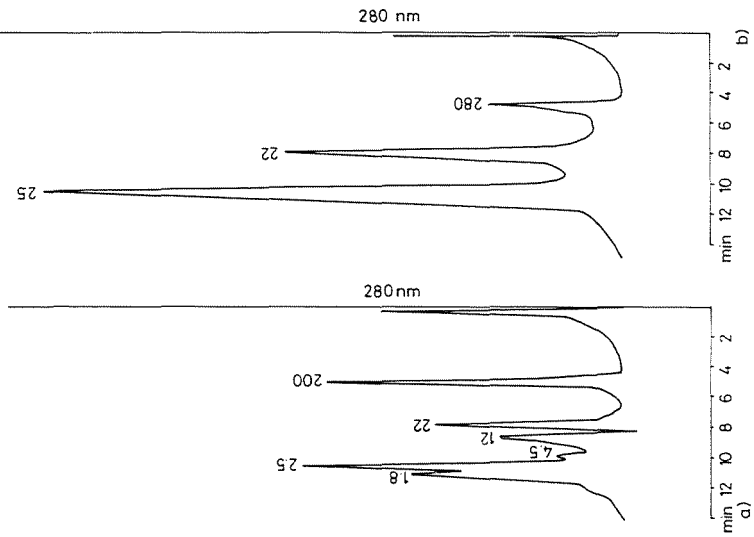

Fig. 3. HPLC separation of albumin
a. MV-SC-550 (waxy), b. SC-6390 HL (opaque-2)

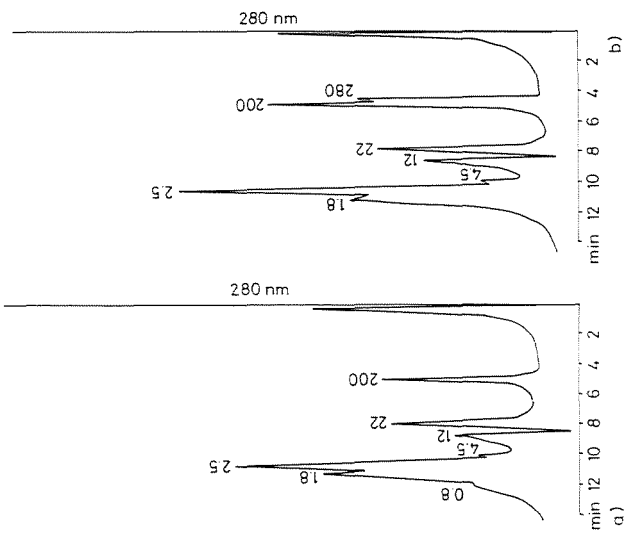

Fig. 2. HPLC separation of albumin
a. MV-SC 394 (flint), b. Pioneer 3732 SC (dent)

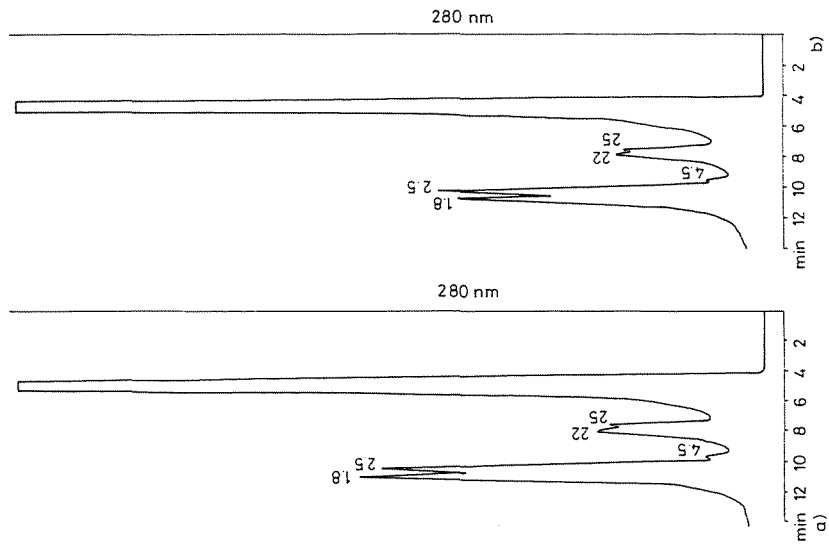

Fig. 4. HPLC separation of albumin
a. White sweet corn, b. Sweet corn MV-SC Ideal

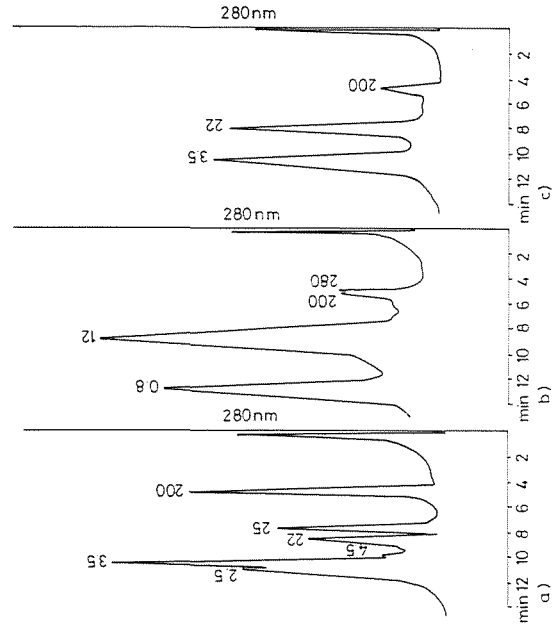

Fig. 5. HPLC separation of albumin
a. Brillant (FRG) b. White corn (USA) c. Giza-2 (Egypt)

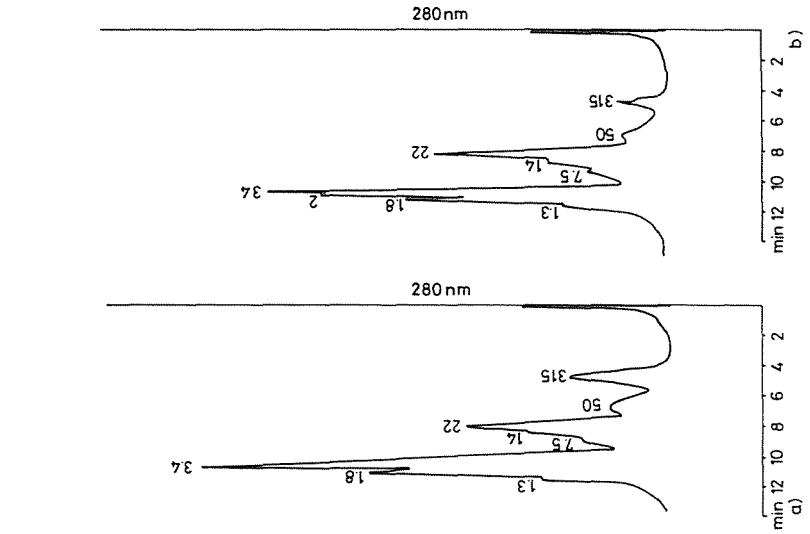

Fig. 6. HPLC separation of globulin
a. MV-SC 394 (flint), b. Pioneer 3732 SC (dent)

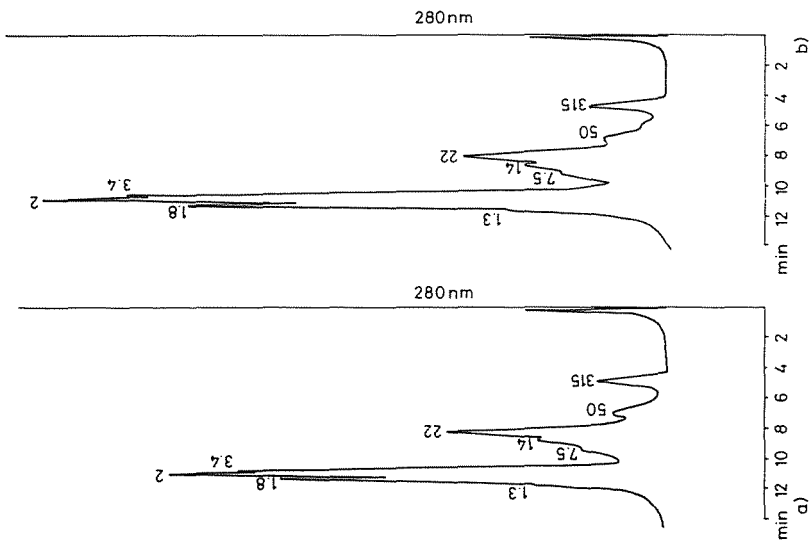

Fig. 7. HPLC separation of globulin
a. MV-SC 550 (waxy), b. SC-6390 HL (opaque-2)

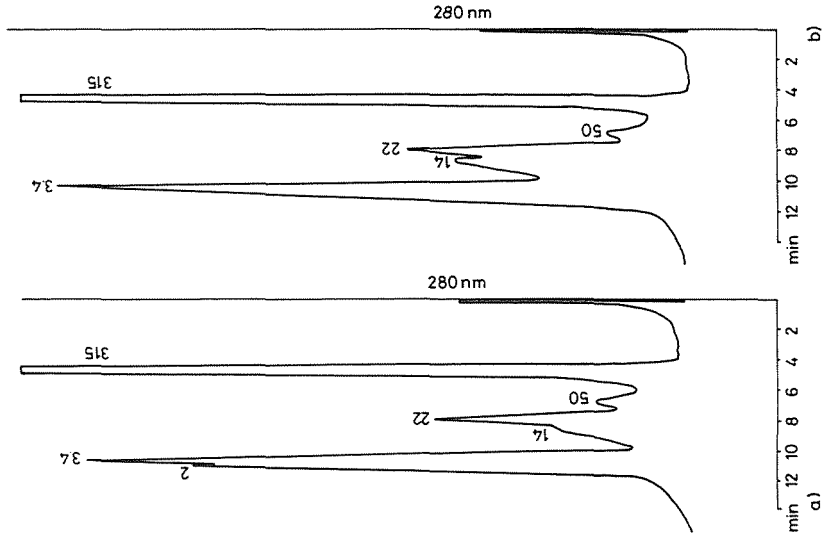

Fig. 8. HPLC separation of globulin
a. White sweet corn, b. Sweet corn MV-SC-Ideal

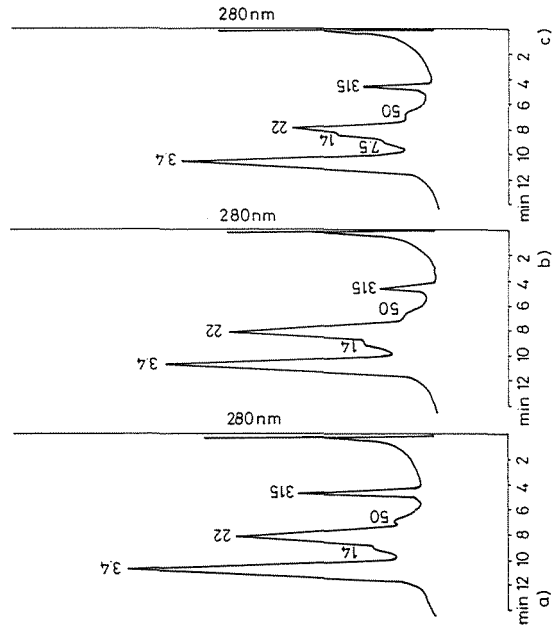

Fig. 9. HPLC separation of globulin
a. Brillant (FRG), b. White corn (USA), c. Giza-2 (Egypt)

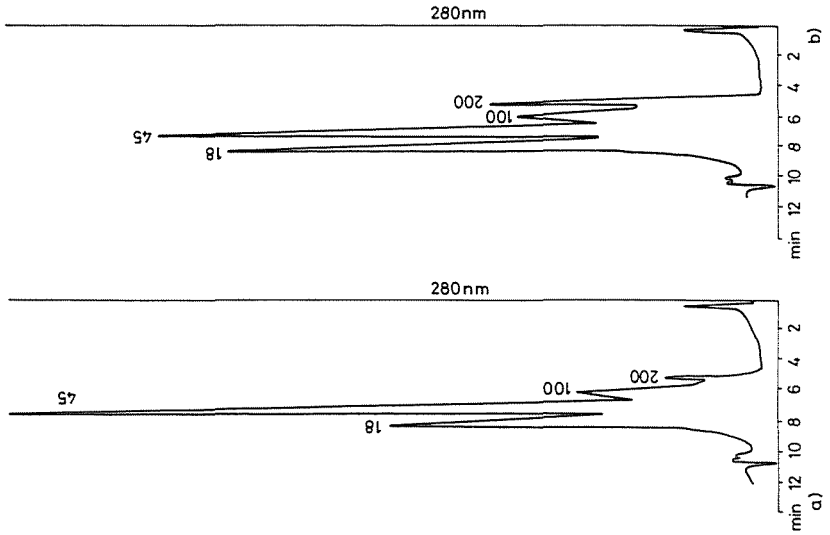

Fig. 10. HPLC separation of zein
a. MV-SC 394 (flint), b. Pioneer 3732 SC (dent)

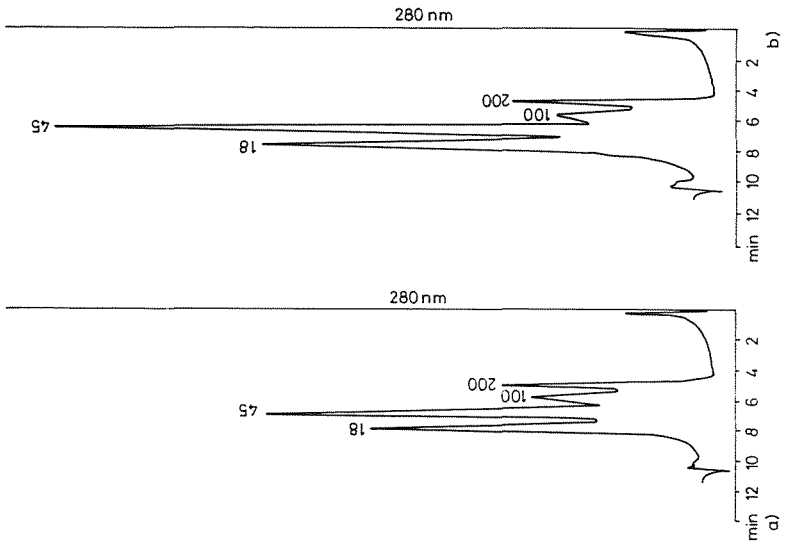

Fig. 11. HPLC separation of zein
a. MV-SC 550 (waxy), b. SC-6390 HL (opaque-2)

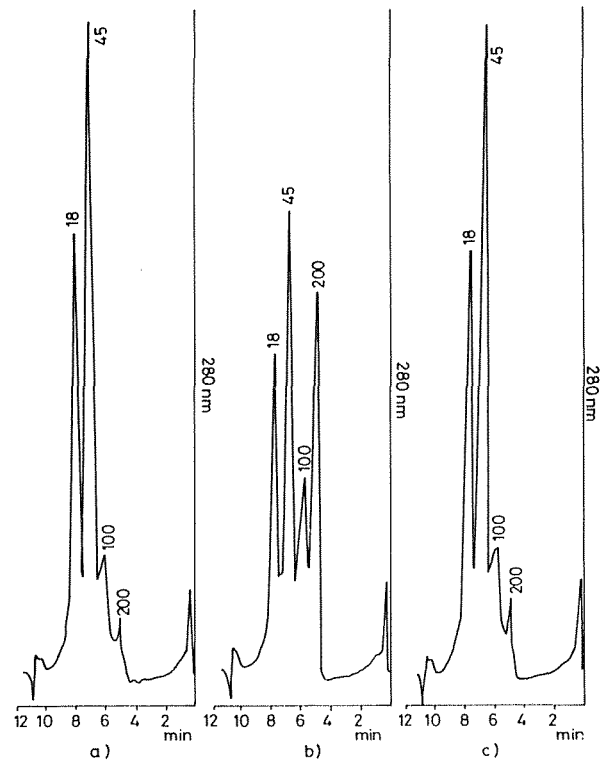

Fig. 13. HPLC separation of zein
a. Brillant (FRG), b. White corn (USA), c. Giza-2 (Egypt)

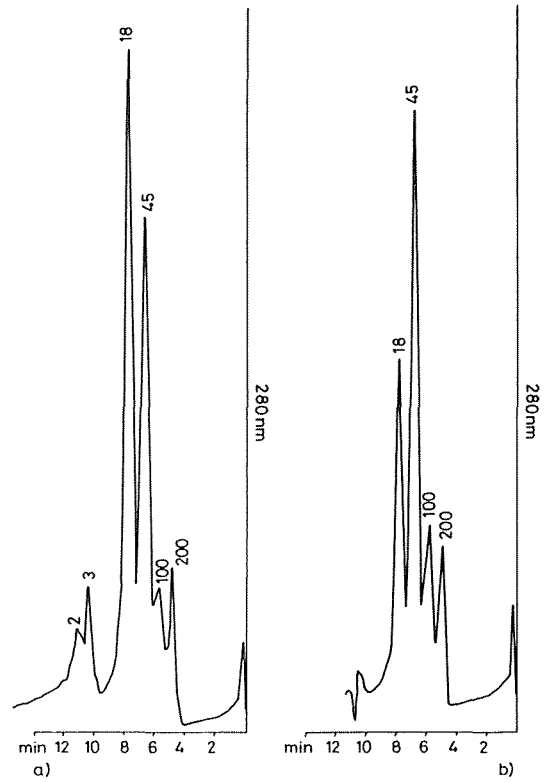

Fig. 12. HPLC separation of zein
a. White sweet corn, b. Sweet corn MV-SC Ideal

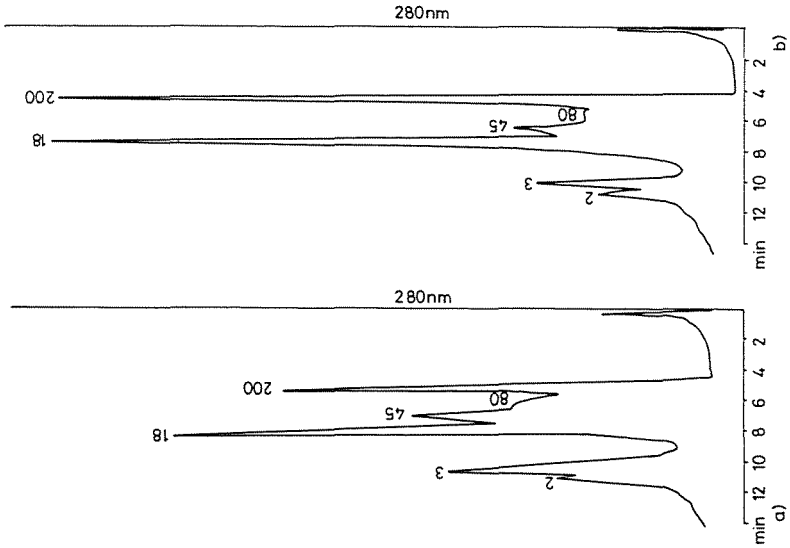

Fig. 14. HPLC separation of glutelin
a. MV-SC 394 (flint), b. Pioneer 3732 SC (dent)

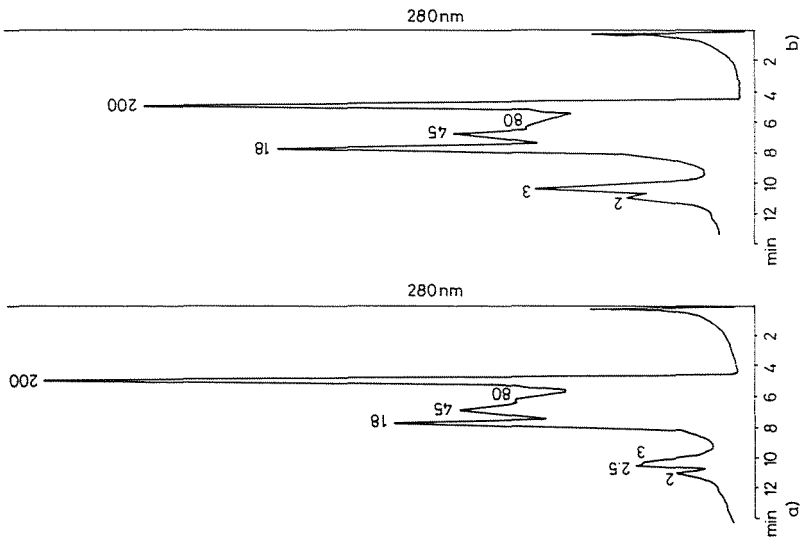

Fig. 15. HPLC separation of glutelin
a. MV-SC 550 (waxy), b. SC-6390 HL (opaque-2)

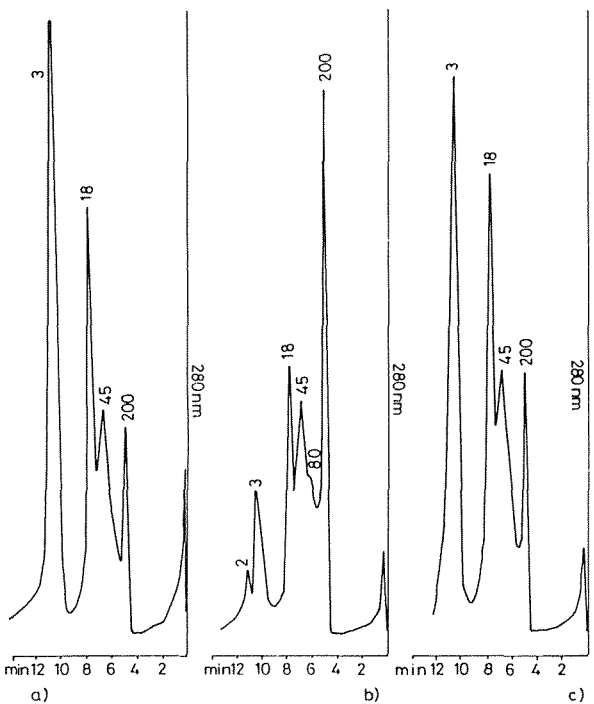

Fig. 17. HPLC separation of glutelin
a. Brillant (FRG), b. White corn (USA), c. Giza-2 (Egypt)

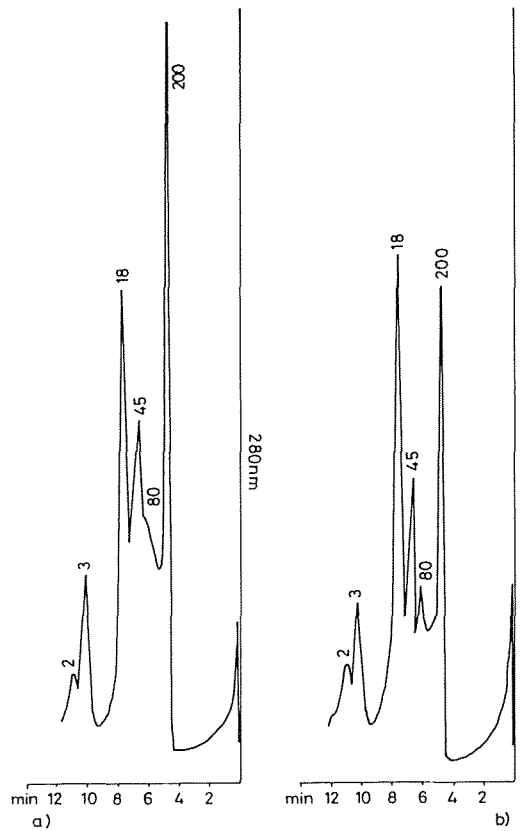

Fig. 16. HPLC separation of glutelin
a. White sweet corn, b. Sweet corn MV-SC Ideal

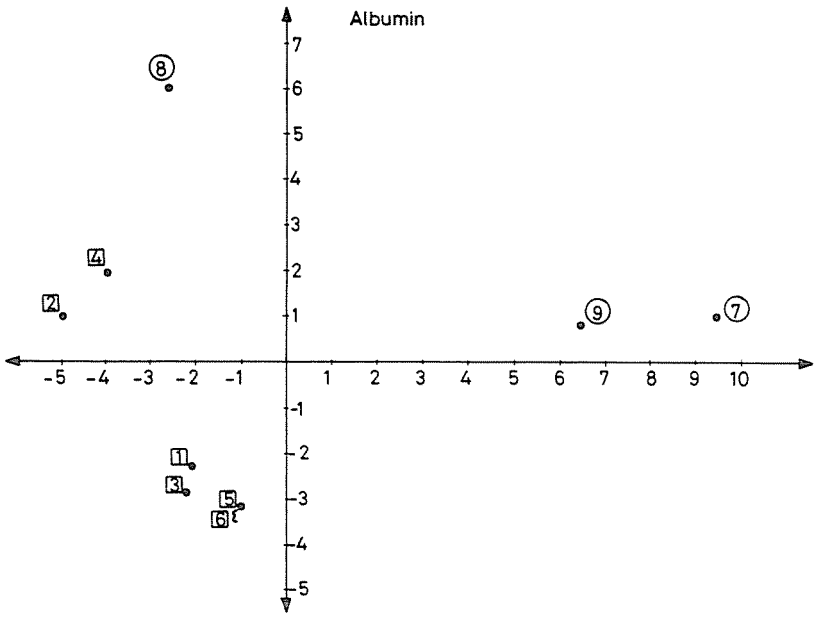

Fig. 18. Distribution of corn varieties, investigated, according to albumin fractions on the plane of the two principal components (numbers refer to varieties in Table 1.)

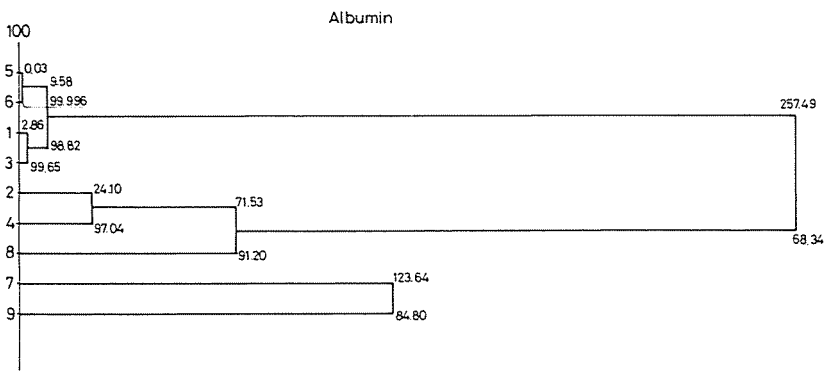

Fig. 19. Dendrogram of corn varieties according to their albumin fractions (numbers refer to varieties in Table 1.)

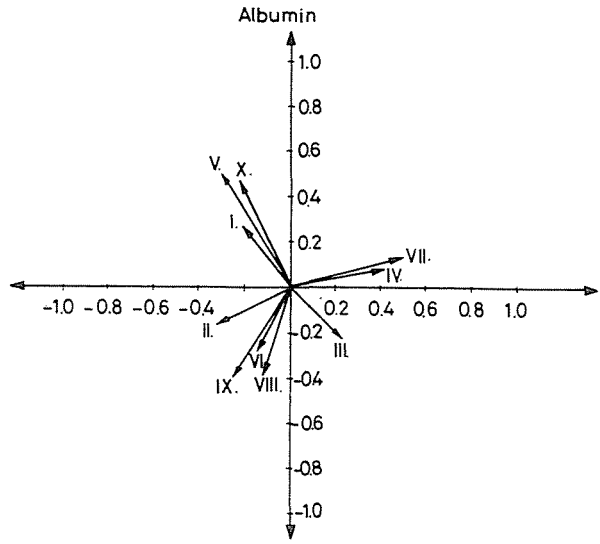

Fig. 20. Projection of original variables (albumin subfractions) on the plane of the first two principal components (for Roman numbers see Table 2.)

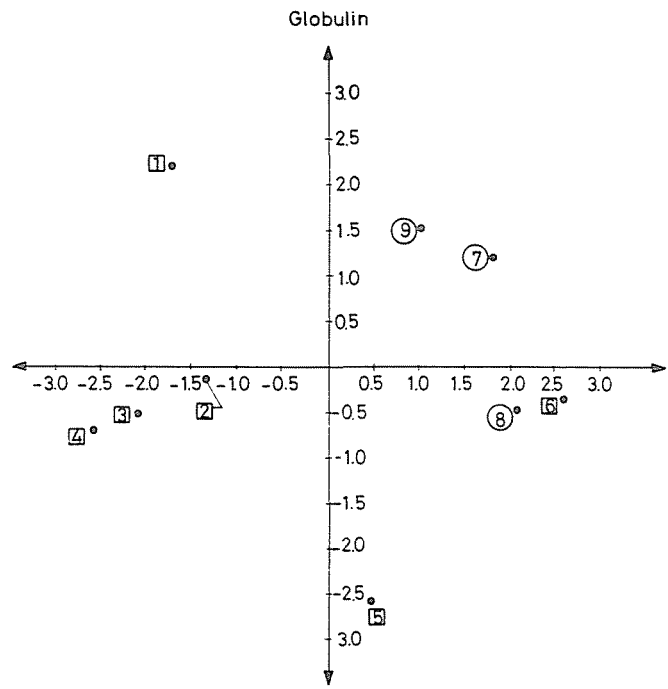

Fig. 21. Distribution of corn varieties, investigated, according to globulin fractions on the plane of the two principal components (numbers refer to varieties in Table 1.)

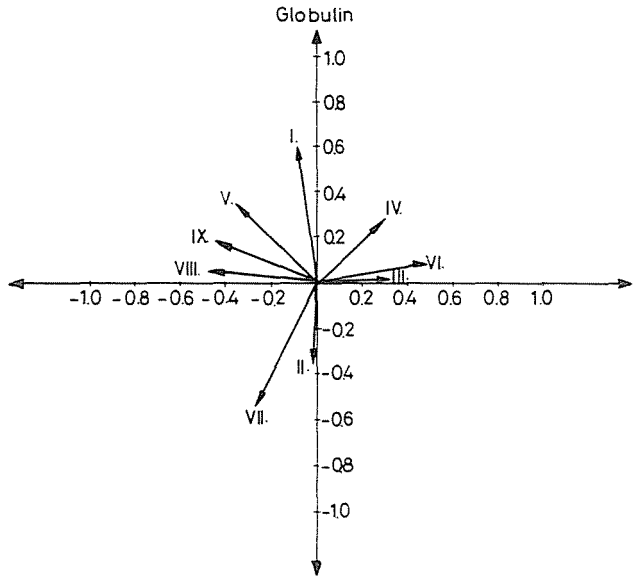

Fig. 22. Projection of original variables (globulin subfractions) on the plane of the first two principal components (for Roman numbers see Table 3.)

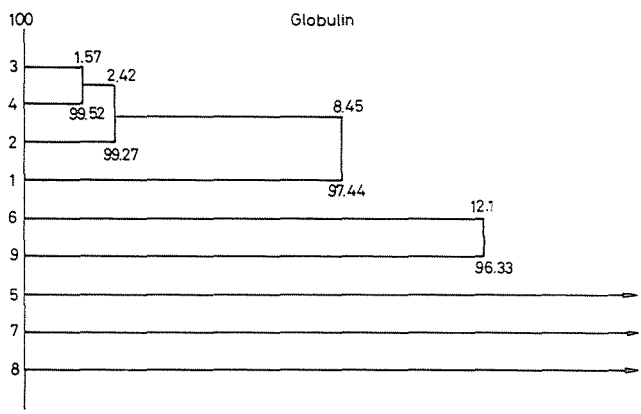

Fig. 23. Dendrogram of corn varieties according to their globulin fractions (numbers refer to varieties in Table 1.)

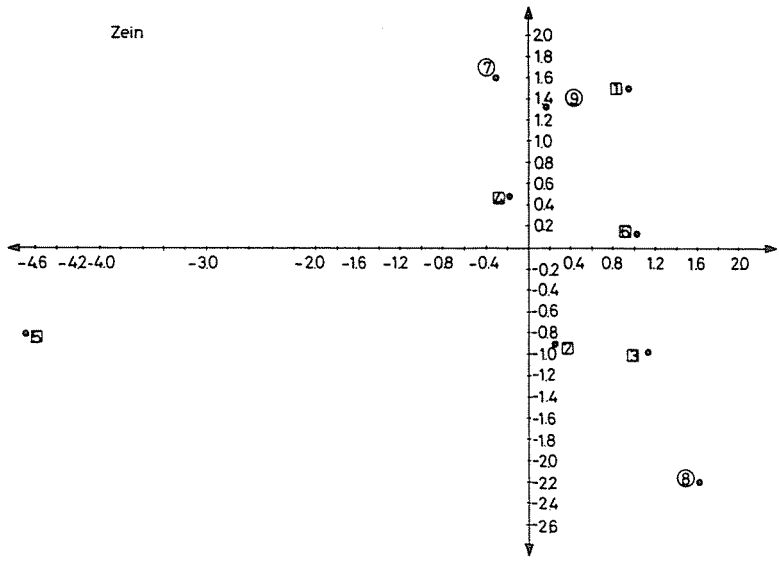

Fig. 24. Distribution of corn varieties, investigated, according to zein fractions on the plane of the two principal components (numbers refer to varieties in Table 1.)

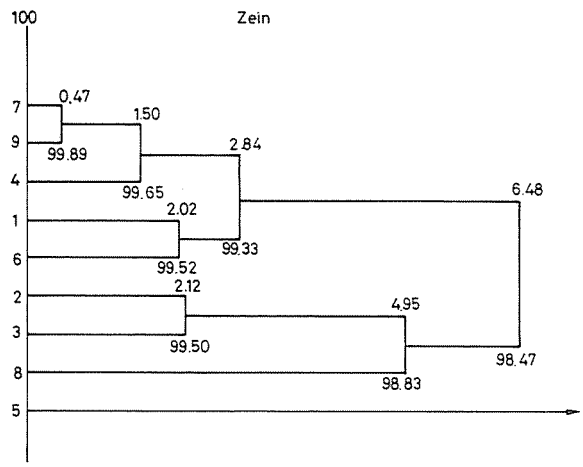

Fig. 25. Dendrogram of corn varieties according to their zein fractions (numbers refer to varieties in Table 1.)

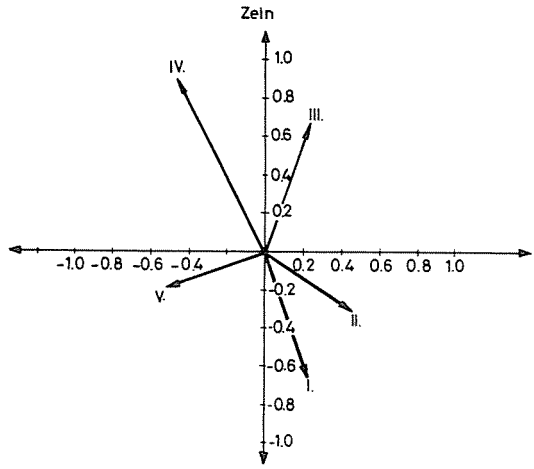

Fig. 26. Projection of original variables (zein subfractions) on the plane of the first two principal components (for Roman numbers see Table 4.)

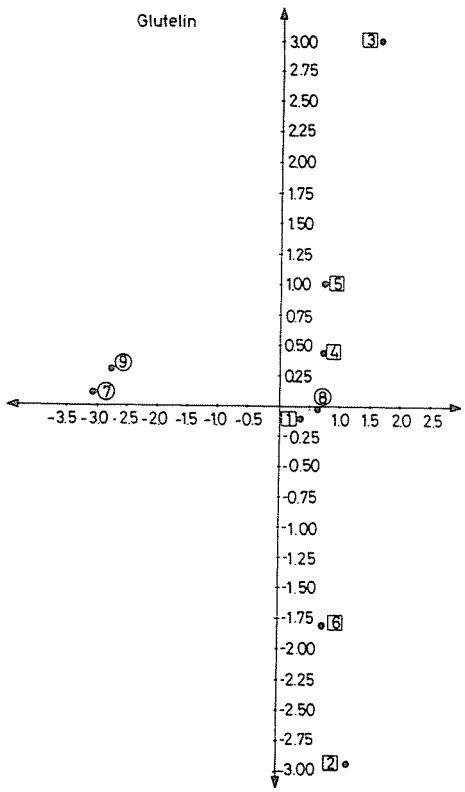

Fig. 27. Distribution of corn varieties, investigated, according to glutelin fractions on the plane of the two principal components (numbers refer to varieties in Table 1.)

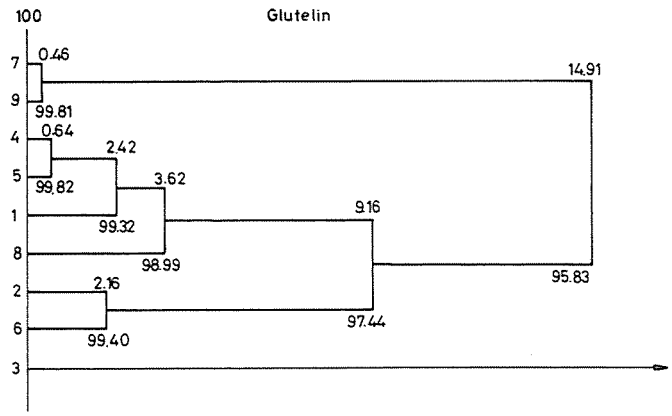

Fig. 28. Dendrogram of corn varieties according the their glutelin fractions (numbers refer to varieties in Table 1.)

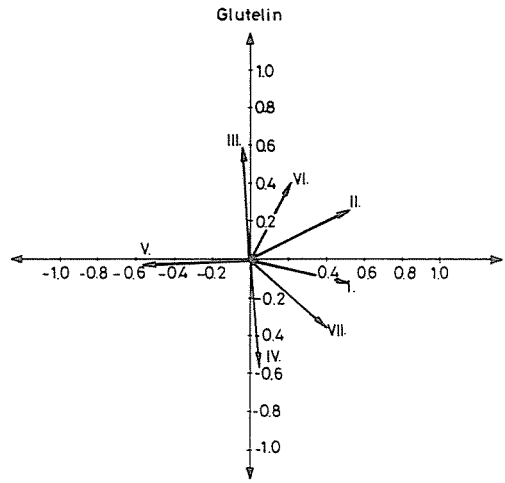

Fig. 29. Projection of original variables (glutelin subfractions) on the plane of the first two principal components (for Roman numbers see Table 5.)

In Fig. 18 corn varieties are plotted in the plane of the first two principal components, calculated from the quantity of fractions obtained from albumin by chromatography.

According to the figure, Hungarian varieties (1, 2, 3, 4, 5, 6) are well separated from the foreign varieties, and are divided into two groups, varieties 2 and 4 can be well separated, and are rather similar to sample 8.

Samples 7 and 9 are sharply separated from all the others, and are primarily characterized by the larger quantities of fractions IV and VII.

Varieties 1, 3, 5 and 6 are characterized by larger quantities of fractions II, VI, VIII and IX while in the case of varieties 2, 4 and 8 the higher quantity of the excluding fractions VI and X and the lower quantity of fraction III is characteristic (see Fig. 20).

The percentage similarity index of corn samples can be read off in accordance with the abovesaid from the dendrogram shown in Fig. 19.

Remarkably, the similarity of corn samples is different in the case of the globulin fraction.

In Fig. 21 corn samples are plotted in the plane of the first two principal components calculated from the quantity of the fractions obtained by HPLC from globulins.

Hungarian samples 1, 2, 3, 4 form according to the dendrograms shown in Figs 22 and 23 a group separated from the other varieties.

The Hungarian variety 5 is not similar to any other sample, while the Hungarian variety 6 resembles on the basis of the globulin fractions foreign varieties. Fractions characteristic of the single groups can be established from Fig. 22, in which the projections of the axes of the original variables are plotted in the plane of the first two principal components.

For varieties 1, 2, 3, 4 mainly the higher quantities of fractions V, VIII and IX, for foreign varieties and Hungarian variety 6 of fractions III, IV, VI and for Hungarian variety 5 of fractions II and VII is characteristic.

The zein fraction shows to a certain degree similar relationships to globulin fractions. In Fig. 24 corn samples are plotted in the plane of the first two principal components, calculated from the quantities of protein fractions, obtained from the zein fraction by HPLC.

Here too, the particular position of Hungarian sample 5 and the similarity of samples 7 and 9 are characteristic. Corn samples 1, 4 and 6 form with the latter a common group, while samples 2 and 3 are more similar to sample 8. For this latter group particularly the higher quantities of fractions I and VI are characteristic.

The group 7, 9 and 1 is characterized by the larger quantities of fractions IV and III and the smaller quantity of fraction V. Sample 5 contains fraction V in the highest quantity.

In Fig. 27 corn samples are plotted in the plane of the first two principal components, calculated from the quantities of fractions obtained by HPLC from glutelin. The characteristic similarity of samples 7 and 9 is present also in this fraction, but sample 3 reveals very different characteristics. Samples 1, 4, 5 and 2, 6 are also here similar, and sample 8 participates again in the group.

The separating samples 7 and 9 are primarily characterized by the higher quantity of fraction V. For samples 4, 5 higher quantities of fractions II and VI, while for samples 2, 6 of fractions IV and VII are characteristic.

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