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An Improved Method for Determination of the Amino Acid Composition in Bulk Protein of Individual Maize Kernels

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The distribution of the amino acids in different parts of single kernels of a selfpollinated inbred line ($W64Ao_2$) of maize was determined. With the method described in the paper it is possible to determine with a high accuracy the amino acid composition from a part of a single kernel without interfering with its viability. The latter is a prerequisite for selection on single-kernel basis.

In plant breeding a special problem arises when the biochemical quality of the product is concerned. In order to determine the amino acid composition of plant seeds one generally has to destroy the seeds and therefore they cannot be used for propagation. This is of particular importance in mutation plant breeding, especially of cross fertilized species, when only one or a few seeds of the same genotype are available.

The technique described in the present paper has the following advantages: (1) it is possible to determine accurately the amino acid composition in small samples of plant material, excised in a representative way, without interfering with the viability of the seed; (2) the short time needed for analysis makes the method feasible for screening procedures; and (3) it is possible to avoid evaporation of the hydrolysate, *i.e.* to save the time needed for this step of analysis and to avoid possible losses in certain amino acids during the evaporation.

MATERIAL AND METHODS

Kernels from one self-pollinated inbred line $W64Ao_2$ of maize were analyzed. A certain part (about 30 mg) of the seed was cut in very thin slices with a razor blade. The cut part was placed into a Pyrex test tube (200 × 8 mm) and dried overnight in a vacuum desiccator (10 mm Hg; concentrated sulfuric acid; sodium hydroxide as pellets). The weight of the sample was determined and 6 N redistilled HCl was added to get 10 mg dry weight material per ml. The sample was then frozen in a beaker with methylcellosolve (or acetone) and dry ice. The tubes were evacuated and sealed. During this procedure, as well as during the opening of the test tubes, it is advisable to wear protective glasses due to the hazard of implosion, which did not happen, however, in our

experience. The test tubes were placed for hydrolysis into an oven at 107°C for 24 h and then cooled to room temperature. The tubes were mixed to get an even distribution of the hydrolyzed sample, and opened. The black humus-like material was allowed to settle for 20—30 min and 50 µl aliquots of hydrolyzate were blown into 2 ml vials containing 1 ml of 0.2 N NaOH, and 2 µl of thiodiglycol. In order to reduce any methionine sulfoxide into methionine, the mixture was incubated at 37°C for 30 min. The samples were kept frozen at −20°C until they were analyzed. Amino acid compositions were determined in a Beckman 120 C amino acid analyzer equipped with a scale expander in the recorder and a long light path flow cell. The method described by Hubbard [1] was followed except that the benzyl alcohol and propanol contents of the B-buffer were 1.5 vol. % and 3 vol. %, respectively.

The signal of the photocells was fed through an integrator (Infotronics) giving the area under each peak which was then compared to standard values. The data were normalized to dry weight and to the total amount of all amino acids.

RESULTS AND DISCUSSION

In Table 1 is shown the variability that arises at the level of the instrument. For most amino acids, except arginine, valine, and methionine, the coefficient of variation is below 5 %. The higher variabilities of the arginine and valine values are due to bad time-programming of the analyzer and the

Table 1. Reproducibility of the determination of the amino acid composition of single maize kernels.[a]

Amino acid	In percent of dry matter			In percent of total amount of amino acids			Number of counts per chromatogram (in hundreds)		
	$\bar{x}$	σ	C.V. %	$\bar{x}$	σ	C.V. %	$\bar{x}$	σ	C.V. %
Lysine	0.418	0.015	3.59	2.90	0.030	1.04	304	13.4	4.42
Histidine	0.412	0.017	4.13	2.85	0.031	1.09	256	10.6	4.13
Arginine	0.643	0.035	5.44	4.45	0.078	1.75	335	13.2	3.93
Aspartic acid	1.293	0.058	4.99	8.97	0.090	1.00	965	27.4	2.84
Threonine	0.522	0.016	3.06	3.62	0.018	0.50	416	16.6	3.40
Serine	0.700	0.035	5.00	4.85	0.065	1.34	674	23.6	3.40
Glutamic acid	3.038	0.086	2.83	21.08	0.084	0.40	1802	40.0	2.22
Proline [b]	1.258	0.033	2.62	8.73	0.071	0.81	—	—	—
Glycine	0.492	0.016	3.25	3.41	0.015	0.44	675	38.4	5.69
Alanine	1.050	0.047	4.48	7.28	0.068	0.93	1059	53.8	5.08
Valine	0.620	0.042	6.77	4.30	0.111	2.58	561	37.4	6.67
Methionine	0.263	0.014	5.32	1.82	0.037	2.03	165	5.1	3.08
Isoleucine	0.490	0.017	3.47	3.40	0.044	1.29	380	15.4	4.05
Leucine	1.833	0.049	2.67	12.72	0.074	0.58	1404	21.0	1.49
Tyrosine	0.610	0.018	2.95	4.23	0.044	1.04	346	16.8	4.87
Phenylalanine	0.763	0.033	4.32	5.29	0.068	1.28	445	12.8	2.87
Mean value	—	—	4.02	—	—	1.13	—	—	3.88

[a] Part of the maize kernel was analyzed in the way described in Materials and Methods. The same hydrolysate was analyzed six times. From obtained data the mean value ($\bar{x}$), standard deviation (σ), and coefficient of variation (C.V.) were calculated.

[b] Peak areas for proline calculated by hand.

integrator, respectively, which can be improved. The higher variability of methionine is probably due to its lower abundance and partly due to its oxidation into methionine sulfone. The low variability values for proline may be due to its relatively high concentration in the hydrolyzate and to the fact that they were here calculated by hand, which eliminates errors from the automatic integration.

When the data are expressed as per cent of total amount of all amino acids a lower variability was observed. For most amino acids, except valine and methionine, coefficients of variation less than 2 % were found.

Since the size of the sample is standardized it is very easy to calculate the amount of each amino acid in the analyzed sample (less than one minute per amino acid). Furthermore, it is possible to use integrated data directly. These data show mostly a somewhat lower variability than data calculated using the standard values (Table 1).

From the same data it is possible to conclude that with increased mean value the standard deviation was increased, but the coefficient of variation

Table 2. Amino acid composition of the bulk protein in different parts of a single maize kernel. Tryptophan is destroyed during hydrolysis. Values for cystine are not reproducible since the samples were not oxidized with performic acid.

Analyzed part [a]								
Amino acid	In percent of dry material	In percent of total amount of amino acids	In percent of dry material	In percent of total amount of amino acids	In percent of dry material	In percent of total amount of amino acids	In percent of dry material	In percent of total amount of amino acids
Lysine	1.23	7.26	0.35	3.41	0.26	3.53	0.23	3.47
Histidine	0.58	3.42	0.36	3.50	0.28	3.81	0.27	4.08
Arginine	2.16	12.76	0.30	2.92	0.29	3.95	0.28	4.23
Aspartic acid	2.02	11.93	0.98	9.54	0.69	9.39	0.62	9.37
Threonine	0.62	3.66	0.38	3.70	0.27	3.67	0.25	3.78
Serine	0.87	5.14	0.50	4.87	0.37	5.03	0.32	4.83
Glutamic acid	2.72	16.07	2.35	22.88	1.77	24.08	1.56	23.56
Proline	1.00	5.91	0.79	7.69	0.68	9.25	0.69	10.42
Glycine	1.00	5.91	0.42	4.09	0.31	4.21	0.27	4.08
Alanine	1.03	6.08	0.72	7.01	0.47	6.39	0.40	6.04
Valine	0.89	5.26	0.59	5.74	0.42	5.71	0.38	5.74
Methionine	0.27	1.59	0.16	1.56	0.10	1.36	0.09	1.36
Isoleucine	0.51	3.01	0.37	3.60	0.25	3.40	0.23	3.47
Leucine	1.03	6.08	1.19	11.59	0.78	10.61	0.69	10.42
Tyrosine	0.39	2.30	0.33	3.21	0.18	2.45	0.17	2.57
Phenylalanine	0.61	3.60	0.48	4.67	0.23	3.13	0.17	2.57
Total amount of amino acids	16.93	100.00	10.27	100.00	7.35	100.00	6.62	100.00

[a] Shadowed part was analyzed.

was decreased. This means that variability occurring in the determination of some amino acids can be reduced by increasing the aliquot size.

Table 2 shows the amino acid composition of the bulk protein in different parts of indivudal maize kernels. The shadowed parts of two single kernels were analyzed as described in Materials and Methods, except that the sample size was not standardized.

The results show that amino acids are distributed uneavenly in different parts of the kernel. As expected, a higher concentration of all amino acids was found in the embryo than in the endosperm. The "top" of the kernel has a greater amount of amino acids than the "sides".

Further experiments were performed to find the most representative part of the kernel with respect to the amino acid content. It has been shown [2] that the representativeness and reproducibility sufficient for the identification of seeds mutated with respect to protein quality can be achieved. In the single backcross of the heterozygote to the opaque mutant $(+/o_2 \times o_2/o_2)$ opaque and normal kernels were obtained in the same ear. It has been shown that the opaque-2 kernels have a considerably higher lysine content [3] than neighbouring normal kernels, *i.e.* that the seed endosperm phenotype is strongly determined by the seed genotype irrespectively of the mother plant genotype. This case is thus similar to the inheritance of oil content in maize,[4] where a considerable part of the inter-seed intra-ear variation was found to be heritable. In such cases a selection on the basis of the analysis of single seeds can be made one generation in advance, *i.e.* with a considerable gain in time and efficiency.

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REFERENCES

1. Hubbard, R. W. *Biochem. Biophys. Res. Commun.* **19** (1965) 679.
2. Denić, M., Dumanović, J., Janovski, I. and Martinović, B. *Vestn. Sel'skokhoz. Nauki. In press.*
3. Mertz, E. T., Bates, L. S. and Nelson, O. E. *Science* **145** (1964) 279.
4. Dumanović, J. and Trifunović, V. *Contemporary Agriculture* (Novi Sad) **1964** 521.

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