

CHANGES IN THE CHEMICAL COMPOSITION AND IN THE DISTRIBUTION OF NITROGEN OF MAIZE AT DIFFERENT STAGES OF DEVELOPMENT¹

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ABSTRACT

On a moisture-free basis, the nitrogen, crude fiber, and ash contents decreased, while the ether extract and carbohydrate content increased with maturation of the corn kernel. The alcohol-soluble nitrogen (zein) increased with development of the grain, the alkali-soluble nitrogen increased at first but decreased 16 days after flowering. The acid-soluble nitrogen of the corn kernel constantly decreased with maturation. The concentration of eight amino acids, in terms of nitrogen of the kernel, was followed during grain maturation. The results indicated that those amino acids found in significant amounts in zein increased, while those found in relatively small amounts in this protein fraction decreased.

In the protein synthesis which accompanies the development of seeds, the amount of protein nitrogen increases at the expense of soluble nitrogen (15-17). Cereal grains are known to contain large amounts of alcohol-soluble protein (2,5,8,9,12,19), and in the mature corn kernel this protein fraction, zein, constitutes about 40 to 50% of the protein nitrogen of the grain (2,8,9,12,17). Several investigators have demonstrated that in the mature corn kernel, alcohol-soluble nitrogen correlates highly with total nitrogen. Furthermore, any changes taking place in the nitrogen of the corn, through breeding (4,6,18) or fertilization (8,18), are reflected in changes in the alcohol-soluble nitrogen of the seed. Except for the work of Zeleny (21), no report has appeared of the time at which the zein is formed in the maturing corn kernel. Zeleny (21) showed that zein is nearly absent in the very immature kernel, but is synthesized at a rapid rate as the grain approaches maturity. He also found that the rapid increase in the ratio of zein nitrogen to total nitrogen is almost exactly paralleled by the decrease in water-soluble nonprotein nitrogen. With the development of a new method of extraction and fractionation of corn proteins by Mertz *et al.* (11,12), it was of interest to study the changes taking place in some nitrogen compounds during the development of the grain.

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Materials and Methods

The seed samples were collected from an open-pollinated corn cultivated on a plot of about 400 sq. meters in the southern coast of Guatemala, at an elevation of 650 ft. The planting consisted of hills spaced 15 in. apart in rows spaced 42 in. apart; each hill contained from one to three plants. The corn was planted in early May 1956 and reached flowering stage in mid-June. Sample collections were begun on June 24 and continued every 7 days until August 18 for a total of nine samples. Every collection contained one ear of corn from each of 15 to 20 randomly selected hills. The samples were collected from 9 to 10 a.m., transported to the laboratory within about 3 hours, and stored at -20°C . Each ear of corn was shelled and the seeds were frozen and ground immediately in a Waring Blendor or Wiley mill. The ground samples were kept frozen at all times, except during the chemical analyses.

Proximate analyses of the grain were carried out as described by the methods of the AOAC (1), using the vacuum-oven method for moisture determinations. Nitrogen was determined in the vacuum oven-dried and wet samples by the Kjeldahl method, selenious acid being used as catalyst; distillation and titration were carried out according to the recommendations of Hamilton and Simpson (7). The method of Mertz *et al.* (11,12) was used for the extraction and fractionation of the nitrogen of the whole ground kernel. For the determination of free ammonia and free amino acids, subsamples of the ground kernel estimated to contain 20 to 30 mg. of dialyzable nitrogen were extracted for 24 hours at 4°C . with 60 ml. of sodium phosphate buffer (pH 6.8–7.0), centrifuged, and the supernatant was collected. The residue was suspended in 25 ml. of water and again centrifuged; the two supernatant solutions were then combined; their volume was measured and the nitrogen content determined by the micro-Kjeldahl method (1). To determine dialyzable nitrogen, 25 ml. of the phosphate buffer extract and of the acid-soluble fraction of the copper sulfate method (11,12) were placed in Cellophane tubing of 19-mm. diameter, surrounded by 50 ml. of the same buffer in the case of the phosphate buffer extract, and by 75 ml. of distilled water in the case of the acid-soluble fraction. After 40 hours at 4°C . the volume of the dialysate was measured and its nitrogen determined. Free ammonia was determined in the phosphate buffer extract by methods of Vickery and Meiss (20), and total free amino acids by the colorimetric method of Moore and Stein (13). Several amino acids were determined microbiologically by means of *Leuconostoc mesen-*

teroides and Difco³ media.

Results

The changes taking place in the proximate chemical composition of the corn kernel during the development of the grain are shown in Table I. No major changes in moisture took place before 16 days

TABLE I
CHANGES IN THE CHEMICAL COMPOSITION OF THE DEVELOPING CORN KERNEL
DETERMINED ON MOISTURE-FREE SAMPLES

DAYS AFTER FLOWERING	MOISTURE	NITROGEN	PROXIMATE ANALYSIS, DRY MOISTURE BASIS				
			Protein ^a	Ether Extract	Crude Fiber	Ash	Carbohy- drates by Difference
	%	%	%	%	%	%	%
10	89.0	2.81	17.6	2.0	9.1	3.7	67.6
16	88.8	2.98	18.6	2.0	8.2	4.3	67.0
23	72.1	1.98	13.3	2.1	5.9	2.7	76.0
30	65.9	1.84	12.3	3.5	4.2	2.3	77.7
37	50.8	1.54	9.6	4.1	3.5	1.7	81.1
44	44.2	1.58	9.9	4.4	3.4	1.6	80.8
51	34.7	1.71	10.7	4.6	3.1	1.5	80.2
58	28.5	1.59	9.9	5.3	2.5	1.3	81.0
65	23.5	1.76	11.0	4.9	3.3	1.3	79.5

^a Percent N \times 6.25.

after flowering. After this period, however, moisture content decreased steadily from 88.8 to 23.5%. The protein content increased slightly from 17.6 at the 10th day to 18.6% on the 16th day, and decreased to 11.0% on the 65th day. The ether-extractable fraction and carbohydrate increased steadily with the development of the grain; crude fiber and ash content decreased up to the 37th day after flowering and remained constant afterwards. The results found here are similar to those reported by Evans (3) on United States corns.

The results of the fractionation of the total nitrogen into acid-, alkali-, and alcohol-soluble (zein) fractions, expressed on a fresh weight basis, are shown in Fig. 1. Acid-soluble nitrogen decreased up to the 30th day and increased thereafter. The decrease was from 73.3% on the 10th day to 20.4% of the total kernel nitrogen on the 30th day. Alkali-soluble nitrogen tended to increase with time. The increase was from 6.4% on the 10th day to 38.6% on the 23rd day, decreasing to 27.6% of the total nitrogen of the grain on the 44th day, with no major change thereafter. The alcohol-soluble nitrogen showed an almost linear increase with the increase in total nitrogen of the samples. The increase was from 11.8 on the 16th day to 41.5%

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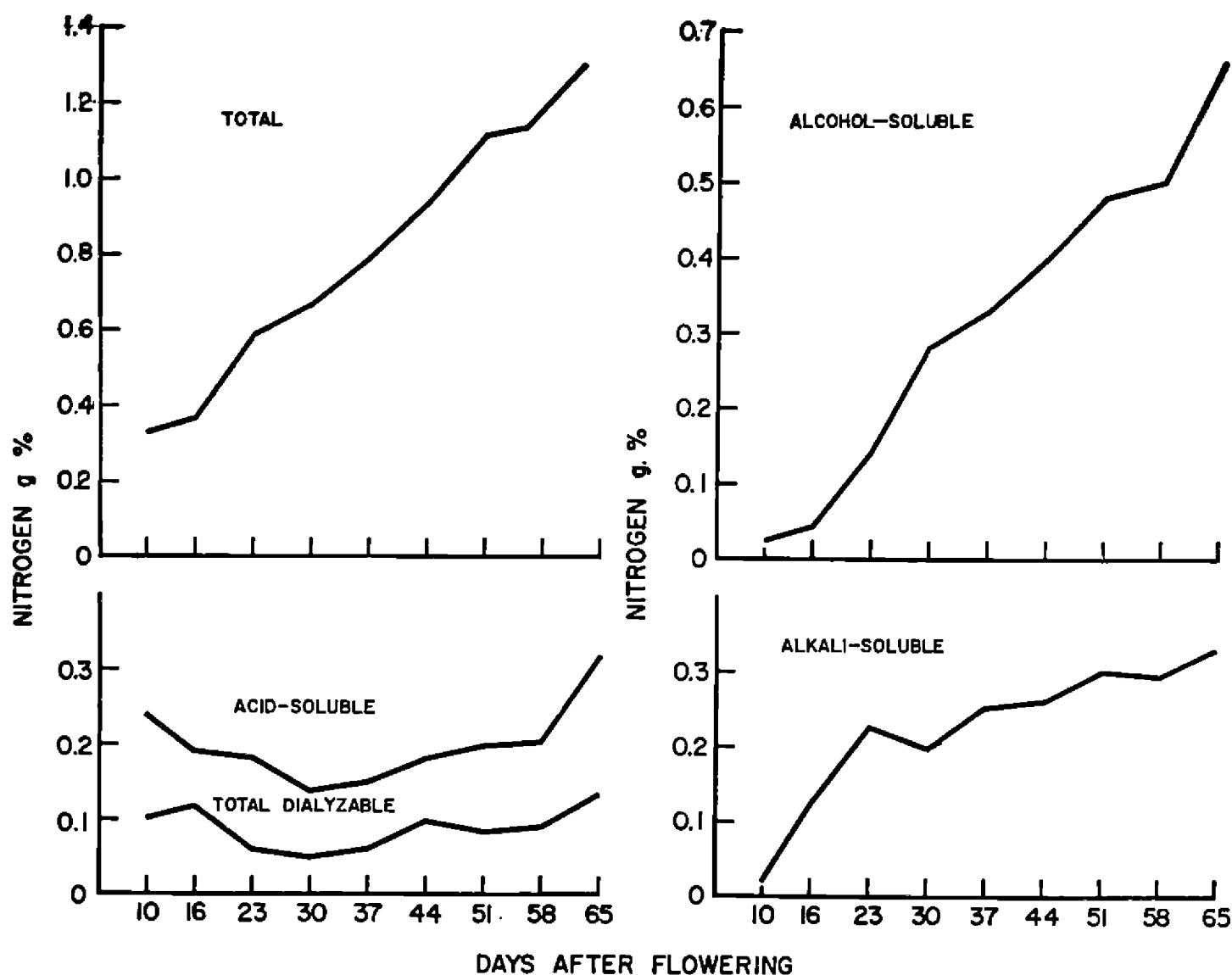


Fig. 1. Changes in nitrogen fraction composition during development of the corn kernel (fresh basis).

on the 30th day after flowering, with subsequent small variations; it reached a value of 50.6% at the end of 65 days.

In the samples collected 10 days after flowering, about 43% of the nitrogen of the acid-soluble fraction was dialyzable. The dialyzable nitrogen of the acid-soluble fraction increased from the 10th to the 16th day, decreased about half by the 23rd day, and remained nearly constant thereafter. When the dialyzable nitrogen of the acid-soluble fraction is expressed as percentage of the total nitrogen extracted from the sample, the same relationship is evident. It decreased from 31.6% on the 10th day to 10.6% on the 23rd day, and remained at about 8.0% up to the 65th day of development.

The amounts of the nitrogen fractions expressed on an absolute basis in the moisture-free samples are shown in Fig. 2. Percent total nitrogen decreased with time of development, but the acid-soluble fraction decreased sharply in about 14 days, while the alcohol-soluble nitrogen increased steadily. The alkali-soluble fraction showed a rise

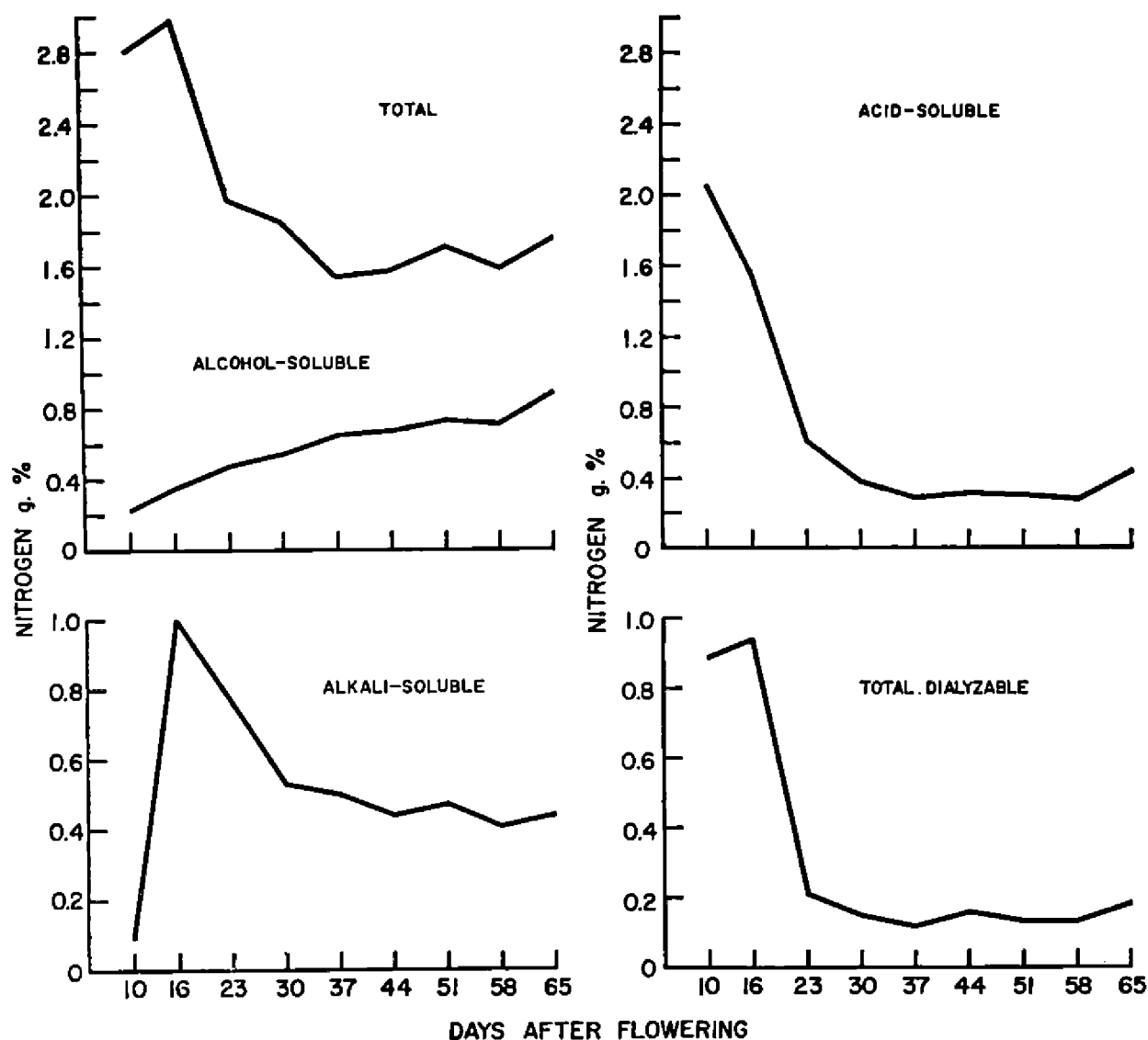


Fig. 2. Changes in nitrogen fraction composition during development of the corn kernel (dry basis).

from the 10th to the 15th day, but decreased thereafter to approximately constant values by the 30th day. Total dialyzable nitrogen varied in the same way.

The total and dialyzable nitrogen contents for the phosphate buffer extracts of the corn samples are shown in Table II. The values are averages of two determinations. The percentages of the total nitrogen extracted with phosphate buffer follow very closely the percentage of total nitrogen present as acid-soluble nitrogen fraction based on the copper sulfate fractionation. The total dialyzable nitrogen as determined from the phosphate buffer extract also follows closely the total dialyzable nitrogen of the acid-soluble fraction obtained by the copper sulfate extraction method (11,12), although the figures are slightly higher in the latter. Also shown in Table II are the values for NH_3 -nitrogen and of the total free amino acids of the phosphate buffer extract. Ammonia nitrogen increased slightly from the 10th to the 16th day, from 1.05 to 1.66%, and decreased

TABLE II
PERCENTAGE OF PHOSPHATE-SOLUBLE AND OF DIALYZABLE NITROGEN, AND CONTENT OF
FREE AMINO ACIDS OF THE CORN KERNEL DURING DEVELOPMENT OF THE GRAIN

DAYS AFTER FLOWERING	SOLUBLE NITRO- GEN IN PHOS- PHATE BUFFER FROM TOTAL KER- NEL NITROGEN	DIALYZABLE NITROGEN IN TOTAL KERNEL NITROGEN	NH ₃ NITROGEN OF TOTAL KERNEL NITROGEN	TOTAL FREE AMINO ACIDS ^a
	%	%	%	g/100 g
10	60.9	30.0	1.05	3.73
16	64.7	34.8	1.66	6.61
23	35.0	15.1	1.00	1.25
30	25.0	10.7	0.59	0.47
37	21.3	8.7	0.36	0.18
44	16.1	6.4	0.25	0.18
51	12.9	4.9	0.36	0.15
58	20.0	5.5	0.21	0.15
65	19.7	4.8	0.11	0.09

^a Equivalent to leucine and expressed on a moisture-free basis.

rapidly thereafter. The total free amino acids of the phosphate buffer extract, expressed as equivalent to leucine, increased from 0.41 g.% on the 10th day to 0.74 g.% on the 16th day, decreasing to 0.35 g.% on the 23rd day. The amount of total free amino acids decreased further, reaching an average of 0.09 g.% after the 30th day post-flowering.

Some of the amino acids of the total proteins of corn and the niacin of the grain were also determined in the different samples and the results are given in Table III. All amino acids studied increased

TABLE III
AMINO ACID CHANGES OCCURRING DURING THE DEVELOPMENT OF THE CORN KERNEL
(Moisture-Free Basis)

DAYS AFTER FLOWERING	ARGININE	ISOLEUCINE	LEUCINE	LYSINE	METHIONINE	PHENYLALANINE	TYROSINE	TRYPTOPHAN	NICOTINIC ACID
	%	%	%	%	%	%	%	%	mg %
10	0.55	0.45	0.82	1.00	0.64	0.45	0.45	0.109	6.73
16	0.89	0.63	1.07	1.16	0.71	0.54	0.45	0.134	10.63
23	0.54	0.50	0.86	0.68	0.39	0.57	0.32	0.065	7.03
37	0.45	0.49	1.30	0.47	0.26	0.55	0.24	0.041	3.92
51	0.47	0.46	1.41	0.44	0.17	0.58	0.28	0.043	2.68
65	0.60	0.55	1.58	0.39	0.16	0.65	0.27	0.043	1.91
	g/g N	g/g N	g/g N	g/g N	g/g N	g/g N	g/g N	mg/g N	
10	0.19	0.16	0.26	0.32	0.21	0.16	0.14	36.4	
16	0.27	0.20	0.31	0.35	0.21	0.15	0.13	40.5	
23	0.25	0.24	0.41	0.32	0.18	0.27	0.14	30.5	
37	0.28	0.30	0.81	0.29	0.16	0.35	0.16	25.3	
51	0.28	0.27	0.82	0.26	0.10	0.34	0.16	25.0	
65	0.35	0.32	0.92	0.23	0.09	0.38	0.16	25.2	

when expressed as percentage of the dry sample, from the 10th to the 16th day. Thereafter, however, and when expressed on the basis of the nitrogen of the sample, the change in the amino acid value patterns differed. Lysine, an amino acid in which zein is notably deficient, decreased from 0.32 to 0.23 g. per g. of nitrogen from the 10th to the 65th day. Methionine also decreased. Isoleucine increased from 0.16 to 0.32 g. per g. of nitrogen from the first to the last of the samples collected. Leucine, present in large amounts in zein, increased almost fourfold from the 10th to the 65th day after flowering, paralleling the increase in zein as the kernel matures. Phenylalanine and arginine doubled in concentration during the maturation of the corn. Tyrosine, on the other hand, after a small initial increase, remained constant. The tryptophan content of the corn kernel increased slightly from the 10th to the 16th day, decreasing again to constant values beyond the 23rd day after flowering.

Because of the recognized relationship of tryptophan to niacin (14), the behavior of the vitamin during development of the grain was investigated. When the niacin is expressed on a moisture-free basis, the values rose from 6.70 to 10.58 mg. per 100 g. in 6 days, decreasing to 7.03 mg.% on the 23rd day and still further with increasing time of maturation of the grain.

No relationship between the values of niacin and tryptophan was apparent, although the maximum values for both occurred, at the same stage of maturation, on the 16th day after flowering. In general, the amino acids which are found in significant amounts in the alcohol-soluble protein of corn, zein, tend to increase with the maturation of the grain, as was the case with the alcohol-soluble nitrogen fraction, while those amino acids which are found in relatively small amounts in zein tend to decrease with time of development of the corn kernel.

Discussion

The results obtained are in agreement with those of Zeleny (21), although a different extraction and fractionation technique was used to determine the changes in the distribution of the nitrogen fraction of the corn kernel as the grain matures. Zein, the alcohol-soluble nitrogen fraction, was nearly absent in the immature kernel, becoming, however, the most important protein fraction as the grain develops and reaches maturity. This increase appears to take place at the expense of the acid-soluble nitrogen either directly or through the dialyzable nitrogen of the acid-soluble nitrogen fraction, both of which behave similarly as the grain matures. From the viewpoint of

the nutritional quality of the proteins in corn, it becomes evident that it would be difficult simultaneously to improve the quantity and quality of the protein of corn; as the nitrogen content of the grain increases, there is a parallel increase in zein which becomes the largest protein fraction of the corn kernel. Similar conclusions have been reached by other workers (4,6,8,9,19). The above statements are also supported by the changes in the concentrations of the amino acids as the grain matures, for, in general, the amino acids present in significant amounts in zein increase while those low in this fraction decrease.

Unfortunately, the amino acids present in zein in significant amounts, for example leucine, are not limiting, while those present in low concentrations, such as lysine, are limiting for animal growth in a large number of cereal grains and other food materials. Since both lysine and tryptophan are low or absent in zein (10) and both showed an initial increase from the 10th to the 16th day after flowering, the changes in concentration of these two amino acids could be related to the changes in the concentration of alkali-soluble fraction as the grain matures, since this nitrogen fraction also showed an increase on the 16th day after flowering. It has already been reported that this fraction contains more lysine and tryptophan than zein (21).

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