Paris, N.; Sauvaire, Y.; Baccou, J. C. Fr. Demande 2315508, 1977 (Appl. 75/20274, 1975).
Payne, P. I.; Corfield, K. G. *Planta* **1979**, *145*, 83–88.
Rafik El-Mahdy, A.; El-Sebaiy, L. A. *Food Chem.* **1982**, *8*, 253–262.
Reichert, R. D.; Mac-Kensie, S. L. *J. Agric. Food Chem.* **1982**, *30*, 312–317.
Sathe, S. K.; Iyer, V.; Salunkhe, D. K. *J. Food Sci.* **1981**, *47*, 8–15.
Sathe, S. K.; Salunkhe, D. K. *J. Food Sci.* **1981**, *46*, 82–87.
Sauvaire, Y.; Baccou, J. C. *Lloydia* **1978a**, *41*, 247–256.
Sauvaire, Y.; Baccou, J. C. *Lloydia* **1978b**, *41*, 588–596.
Sauvaire, Y.; Baccou, J. C.; Besançon, P. *Nutr. Rep. Int.* **1976**, *14*, 527–537.
Singh, U.; Jambunathan, R. *Qual. Plant.—Plant Foods Hum. Nutr.* **1982**, *31*, 347–354.
Sosulski, F. W. In "Food colloids"; Graham, H. D., Ed.; Avi Publishing Co.: Westport, CT, 1977; Chapter 3, pp 152–206.
Wolf, W. J. In "Advances in Cereal Science and Technology"; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1976; Chapter 6, pp 325–377.
Wolzak, A.; Elias, L. G.; Bressani, R. *J. Agric Food Chem.* **1981**, *29*, 1063–1068.
Wunschendorff, M. H. E. *J. Pharm. Chim.* **1919**, *20*, 86–88.
Yacoub, J. J.; Aboul-Ela, M. M. *Tex. J. Sci.* **1979**, *31*, 333–341.
Youle, R. J.; Huang, A. H. C. *Plant Physiol.* **1978**, *61*, 13–16.

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Amino Acid Composition of Sorghum Grains As Influenced by Grain Maturity, Genotype, and Nitrogen Fertilization

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Field experiments were conducted in 1977 and 1978 using three sorghum genotypes, namely, RCFA × L. 187 (a hybrid), L.187, and SK 5912, and four nitrogen rates (0, 35, 70, and 140 kg of N/ha). Nitrogen application generally increased total amino acids, g/16 g of N, but consistently decreased total essential amino acids of grains. Essential amino acid concentration significantly ($P = 0.05$) increased with grain maturity in both years. Genotype influenced grain amino acid composition with genotypes ranking in the orders of SK 5912 > RCFA X L.187 > L.187 for valine and SK 5912 > L.187 > RCFA × L.187 for methionine. Inverse relationships were evident between grain protein and lysine and methionine and between the latter two and leaf nitrate reductase activity.

All cereals tend to have an imbalance of the essential amino acids in that they are deficient in three (lysine, tryptophan, and methionine) of the eight amino acids considered indispensable for monogastrics (Breteler, 1976; Frey, 1973), yet the protein in cereals is, for most people, the major part of their protein intake, especially in the developing countries (Munck, 1975). Furthermore, of all cereals, sorghum [*Sorghum bicolor* (L.) Moench] has the lowest biological value because it has very low levels of lysine, tryptophan, and possibly arginine (Eggum, 1973; Sikka and Johari, 1979).

Nitrogen levels supplied to sand culture as solutions of nitrate in concentrations ranging from 0.2 to 16 mequiv/L have been shown to increase the proportions of certain amino acids in sesame (Mitchell et al., 1976). Reports have also indicated that the amino acid composition of grain is probably under genetic control (Nelson et al., 1965; Frey, 1973).

Although the work of Rhodes and Mathers (1974) on barley [*Hordeum vulgare* (L.)] indicated that amino acid composition of grains is affected by grain maturity, there is, however, little information on the amino acid composition of sorghum grain at various stages of grain development. The study reported here was, therefore, undertaken to investigate the pattern of amino acid composition of sorghum grain at different stages of grain development as affected by genotype and soil nitrogen level. The correlations between leaf nitrate reductase activity and grain protein at harvest and lysine and methionine were also determined. The three genotypes employed in this study are being used in cooperative research on grains for human consumption in the West African semiarid regions.

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MATERIALS AND METHODS

Three sorghum genotypes, namely, RCFA × L.187 (a hybrid), L.187, and SK 5912 were field grown on a sandy loam soil at the Agronomy Farm of the Institute for Agricultural Research, Ahmadu Bello University, Samaru, Zaria (11°11′ N and 7°38′ E), Nigeria.

The field received blanket applications of 89.6 kg/ha super phosphate and muriate of potash. These were broadcast and disked in at seed-bed preparation. Seeds of the three genotypes were sown in June on 90-cm ridges at 60 cm apart, giving a plant population of 37000/ha at two plants per stand with uniform plot sizes of 9.28 m by 7.1 m. Four levels of nitrogen, 0, 35, 70, and 140 kg of N/ha, were applied as calcium ammonium nitrate according to AERLS Recommended Practices No. 2 (1976, 1978). The experiments carried out in 1977 and 1978 were a completely randomized block design with treatments replicated 4 times.

Nitrate reductase activity (NRA) of the fourth leaf was measured at 5, 7, 9, 11, 13, and 15 weeks after planting by using a combination of the in vivo methods of Klepper et al. (1971) and Radin (1973).

Grain protein and amino acid composition of the grains were determined at 10, 17, 24, and 67 (at harvest) days after anthesis by the Kjeldahl method and by using a multisample analyzer, Model TSM (Technicon), respectively. The hydrolysis of the amino acids was by 6 N hydrochloric acid in which the tryptophan was destroyed. The readings for cystine were so low that they were dis-

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Table I. Effect of Nitrogen Supply and Stage of Grain Maturity on Total Amino Acid Content of the Genotypes (g/16 g of N)[a]

stage of grain maturity, days after anthesis	treatments, kg of N/ha			
	0	35	70	140
		RCFA × L.187		
10	67.82	91.42	87.08	81.71
17	91.08	78.13	80.27	85.62
24	95.67	95.84	85.82	93.44
67[b]	105.43	115.14	112.96	98.18
		L.187		
10	81.76	85.27	86.24	85.63
17	83.88	77.89	80.74	80.83
24	89.99	92.49	92.16	86.15
67[b]	99.60	105.26	111.00	109.56
		SK 5912		
10	72.29	87.31	91.48	79.45
17	77.66	90.13	81.51	81.22
24	87.47	79.55	86.81	89.43
67[b]	109.93	114.28	114.71	110.95

[a] Sampling data × genotype × treatment LSD 5% = 8.48. [b] At harvest.

Table II. Effect of Nitrogen Supply and Stage of Grain Maturity on Total Essential Amino Acid Content of the Genotypes (g/16 g of N)[a]

stage of grain maturity, days after anthesis	treatments, kg of N/ha			
	0	35	70	140
		RCFA × L.187		
10	32.05	29.02	27.26	23.89
17	31.73	26.30	27.05	29.80
24	38.50	35.21	35.40	34.34
67[b]	43.70	41.47	39.48	37.03
		L.187		
10	32.53	31.83	31.81	31.77
17	29.69	26.63	26.90	26.99
24	33.17	32.29	32.40	30.15
67[b]	40.25	37.92	37.27	37.47
		SK 5912		
10	26.41	30.32	29.26	25.35
17	26.34	30.38	26.64	27.08
24	33.19	28.55	29.97	30.40
67[b]	42.97	42.72	41.12	40.78

[a] Sampling date × genotype × treatment LSD 5% = 2.47. [b] At harvest.

Table III. Effect of Nitrogen Supply and Stage of Grain Maturity on Total Nonessential Amino Acid Content of Genotypes (g/16 g of N)[a]

stage of grain maturity, days after anthesis	treatments, kg of N/ha			
	0	35	70	140
		RCFA × L.187		
10	55.77	62.40	59.82	57.81
17	59.35	50.12	60.23	55.82
24	60.17	60.12	60.17	58.24
67[b]	61.63	72.43	73.07	61.10
		L.187		
10	49.23	53.43	54.81	53.89
17	54.20	51.26	53.84	50.91
24	58.40	60.84	73.73	71.10
67[b]	59.85	69.84	69.78	56.00
		SK 5912		
10	54.88	56.99	62.20	49.00
17	50.33	59.75	54.86	55.45
24	50.33	51.25	56.84	59.03
67[b]	66.96	71.56	73.09	67.63

[a] Sampling date × genotype × treatment LSD 5% = 6.10. [b] At harvest.

carded as they were grossly inaccurate. This acid hydrolysis is what was prescribed for the amino acid analyzer, the best and most accurate available here at the time of the study. Data were subjected to analyses of variance, and correlation coefficients were computed as appropriate by using the Statistical Package for Social Scientists (SPSS). This was the best package we had for analysis of variance regression and correlation analysis at the time. This SPSS is now being gradually replaced by the Genstat package. Nitrate reductase activity at the different sampling dates and grain proteins at harvest were correlated with lysine and methionine, respectively, at harvest to determine the type of relationships that existed among the factors mentioned. All amino acid values reported here are expressed on a gram per 16 g of N basis.

RESULTS

Effect of Nitrogen Supply on Amino Acid Composition. Nitrogen treatment increased total amino acids at first and last sampling dates in both years although this phenomenon was not significant (Table I). Total essential amino acid concentration of sorghum grain decreased consistently but significantly ($P = 0.05$) in RCFA × L.187 and L.187 in both years as levels of applied nitrogen were increased (Table II). The decrease was not as consistent in SK 5912. Conversely, however, the concentration of total nonessential amino acids increased generally with levels of nitrogen application from 0 to 70 kg of N/ha but at 140 kg of N/ha treatment the increase was not as consistent (Table III). In RCFA × L.187, only at 10 and 67 days after anthesis were the values of total amino acid concentration at 35 kg of N/ha significantly higher than the values at 0 kg of N/ha. In L.187 and SK 5912, total amino acid values were highest at 70 kg of N/ha at 10 and 67 days after anthesis (Table I).

Grain Maturity. Total essential amino acids significantly ($P = 0.05$) increased with grain maturity (Table II) except the concentration of a particular amino acid, lysine, which decreased with grain maturity although the decrease was significant only in 1977 (Table IV). Table II also shows that among the three genotypes, SK 5912 generally had the highest total essential amono acid concentration at harvest in the two years of the study. Total essential amino acid concentration of genotypes except SK 5912, was always highest on soil with no additional nitrogen in both years (Table II). This trend was not consistent in SK 5912.

At all sampling dates in both years, and at all nitrogen levels, the essential amino acids with the lowest and highest values were methionine and leucine, respectively. For example, while at harvest in 1977, leucine values were 13.55, 13.72, 13.17, and 12.97 g/16 g of N at 0, 35, 70, and 140 kg of N/ha, respectively, the values for methionine were 2.95, 2.83, and 2.23, and 2.36 g/16 g of N at corresponding nitrogen levels.

In both years and regardless of sampling dates, glutamic acid had the highest values (g/16 g of N) in each of the three genotypes. The amino acid with the second highest values in each genotype was aspartic acid except at 24 and 67 days after anthesis when leucine values were higher. Generally, the three genotypes were especially rich in glutamic acid, leucine, aspartic acid, alanine, and proline in both 1977 and 1978. It was observed at harvest that SK 5912 was superior to the other genotypes in its composition

Table IV. Effect of Grain Maturity on (Individual) Amino Acid Composition of Sorghum Grains (Combined Analysis of Variance over Three Genotypes and Four Nitrogen Levels) in 1977 and 1978[a]

	stages of grain maturity							
	10 days[b]		17 days[b]		24 days[b]		67 days[b,c]	
amino acids	1977	1978	1977	1978	1977	1978	1977	1978
lysine	4.10	4.17	3.46	4.26	3.12	3.96	2.59	3.58
histidine	2.64	2.60	2.43	3.12	2.50	2.82	3.02	4.20
arginine	4.10	4.06	3.68	3.85	3.53	3.43	4.51	5.41
aspartic acid	11.65	13.89	8.67	10.07	8.40	8.52	8.16	8.76
threonine	3.67	3.50	3.24	3.72	3.43	3.79	3.50	4.25
serine	4.16	4.16	3.74	4.42	4.42	4.33	4.85	5.77
glutamic acid	13.99	12.45	15.91	16.23	17.69	17.93	21.27	22.57
proline	5.09	4.70	4.25	4.80	5.78	6.44	7.89	10.10
glycine	4.44	3.81	3.42	3.70	3.32	3.65	3.49	4.20
alanine	6.07	6.49	6.85	8.07	8.35	8.76	9.09	9.99
valine	4.60	4.60	4.34	4.43	4.74	5.30	5.29	6.15
methionine	2.14	2.10	2.15	2.08	2.23	2.62	2.59	2.47
isoleucine	4.10	3.28	3.35	3.28	3.83	3.78	4.33	4.74
leucine	6.72	9.16	6.92	7.71	9.80	9.68	13.35	14.33
tyrosine	3.04	2.93	2.96	3.35	3.24	2.59	3.95	4.80
phenylalanine	3.52	3.68	3.34	3.85	4.58	4.45	5.32	6.38

[a] Amino acid × stage of grain maturity LSD 5% = 0.62 for 1977 and 1.87 for 1978. [b] After anthesis. [c] At harvest.

Table V. Relationship between NRA[a] and Lysine and between NRA and Methionine in the Grains of the Three Genotypes over the Two Years of Study

age of plants, weeks after planting	correlation coefficients[b]					
	RCFA × L.187		L.187		SK 5912	
	NRA vs. Lys	NRA vs. Met	NRA vs. Met	NRA vs. Met	NRA vs. Lys	NRA vs. Met
5	−0.65***[c]	−0.52***	−0.44**	−0.91***	−0.37**	−0.25**
7	−0.33*	−0.38**	−0.58***	−0.65***	−0.29*	−0.57***
9	−0.55***	−0.23	−0.41*	0.20	−0.97***	−0.77***
11	−0.50***	−0.48***	−0.09	−0.40**	0.18	0.36
13	−0.41**	−0.78***	0.28	0.16	−0.40**	−0.08
15	−0.71	−0.57***	−0.46**	−0.56***	−0.49***	−0.36*

[a] NRA = nitrate reductase activity of the fourth leaf. [b] $n = 48$. [c] (*), (**), and (***) = probabilities at 0.05, 0.01, and 0.001, respectively.

of essential amino acids. In both years, this genotype ranked highest in all the essential amino acids, except valine and methionine in 1977 and in phenylalanine in 1978.

Correlations. Nitrate reductase activity (NRA) measured at various stages of plant growth was found to be nearly always negatively correlated with lysine and methionine respectively in each genotype and for the two years of the study combined (Table V). Considering the two years separately, RCFA × L.187 gave the highest correlation between NRA and lysine at 5 weeks after planting ($r = -0.58$ ($P = 0.05$) and -0.65 ($P = 0.05$) for 1977 and 1978, respectively). The same was also true between NRA and methionine at 5 weeks after planting, r being -0.45 and -0.52 in 1977 and 1978, respectively, but none was significant. The correlation of NRA with lysine gave the r values as -0.73 ($P = 0.05$) (1977) and -0.58 (1978) for L.187 at 13 and 7 weeks after planting, respectively. In SK 5912, the highest correlation between NRA and lysine was $r = -0.61$ in 1977 and $r = -0.97$ ($P = 0.01$) in 1978 at 7 and 9 weeks after planting, respectively. The association between NRA and methionine followed the same pattern with $r = -0.68$ ($P = 0.05$) (1977) and $r = -0.77$ ($P = 0.01$) (1978), respectively.

The correlations between grain protein and lysine and between grain protein and methionine, respectively, for the genotypes were also negative although not all of them were significant (Table VI).

DISCUSSION

The increases and decrease in nonessential and essential amino acids, respectively, with increasing nitrogen supply are consistent with earlier reports on wheat and oats (Eppendorfer, 1978). Although total amino acids generally increased with nitrogen supply, there was a decrease at the highest nitrogen treatment of 140 kg of N/ha. This suggests that high nitrogen levels will not always bring about increases in total amino acid content in sorghum grains. Beyond this threshold level, a decline in total amino acid concentration could be expected.

In spite of the inconsistency in the absolute values of amino acids, at 17 and 24 days after anthesis, the values (except those of lysine and aspartic acid) were consistently higher at harvest as compared to those at the first sampling date of 10 days after anthesis (Table IV). Therefore, there appears to be no advantage in harvesting sorghum grain for animal feed or human consumption before maturity as neither amino acids (except lysine and aspartic acid) nor dry matter are maximized by such a practice.

That genotype influenced the amino acid composition of sorghum grain in this study reinforces the reports on

Table VI. Correlation Coefficients in 1977 and 1978 between Lysine and Grain Protein at Harvest and between Methionine and Grain Protein at Harvest

	correlation coefficients			
	grain protein and lysine		grain protein and methionine	
genotype	1977	1978	1977	1978
RCFA × L.187	−0.30	−0.56*[a]	−0.01	−0.03
L.187	−0.84**[b]	−0.03	−0.51	−0.27
SK 5912	−0.60*	−0.56*	−0.08	−0.53

[a] (*) = probability at 0.05. [b] (**) = probability at 0.01.

corn [(*Zea mays* (L) (Nelson et al., 1965)] and barley (*H. vulgare*) (Eppendorfer, 1978).

Although SK 5912 was easily the highest in its concentration of essential amino acids, the other two genotypes did not clearly differ in this regard. For example, the ranking of genotypes for individual essential amino acids gave the following orders: SK 5912 > RCFA × L.187 > L.187 for lysine and leucine, while it was SK 5912 > RCFA × L.187 > L.187 for valine and SK 5912 > L.187 > RCFA × L.187 for methionine consistently in both years (unpublished data).

The negative *r* values obtained for correlations of NRA with either lysine or methionine as well as correlation of grain protein at harvest with either of these amino acids show some inverse or antagonistic relationships among these factors. Similar reports were given by Eppendorfer (1975). This negative relationship could be due to much larger increases in the prolamin and partly glutelin fractions of grain protein than the albumin and globulin fractions. This would then result in a depression in the concentrations of lysine and methionine in the total grain protein (Eppendorfer, 1975). This explanation is further supported by the general increase in total amino acids with increase in nitrogen levels in this study whereas total essential amino acids decreased (Table I).

CONCLUSIONS

High nitrogen levels would tend to decrease the essential amino acids of sorghum grains on a grams per 16 g of N basis. Except for lysine and aspartic acid, amino acid values of sorghum grains were highest at harvest. There is, therefore, not much nutritional advantage in harvesting sorghum grains for human consumption before grain maturity. Of the three genotypes tested in this study, SK 5912 was the highest in amino acid composition. The genotype is, therefore, a possible parent line where breeding for high protein and amino acids in sorghum is the object of study.

Inverse relationships were evident between grain protein and lysine, grain protein, and methionine, between NRA and lysine, and also between NRA and methionine. The three genotypes were rich in glutamic acid, aspartic acid, leucine, alanine, and proline. They had moderate amounts of phenylalanine, arginine, histidine, tyrosine, and isoleucine but were very low in lysine and methionine. Methionine was the lowest of all the amino acids and in each of the genotypes studied.

Registry No. Valine, 72-18-4; methionine, 63-68-3; lysine, 56-87-1; nitrate reductase, 9013-03-0.

LITERATURE CITED

AERLS "Recommended Practices No. 2"; Agricultural Extension and Research Liaison Services, Ahmadu Bello University: Zaria, Nigeria, 1976.

AERLS "Recommended Practices No. 2"; Agricultural Extension and Research Liaison Services, Ahmadu Bello University: Zaria, Nigeria, 1978.

Breteler, H. *J. Sci. Food Agric.* **1976**, *27*, 978–982.

Eggum, B. O. In "Nuclear techniques for seed protein improvement"; I. ALA: Vienna, 1973; pp 39–408.

Eppendorfer, W. H. "Proceedings of the 11th Colloquium of the International Potash Institute, Ronne, Bornholm, Denmark"; International Potash Institute, CH-3048: Worblanfen-Berne, Switzerland, 1975; pp 249–260.

Eppendorfer, W. H. *J. Sci. Food Agric.* **1978**, *29*, 995–1001.

Frey, K. J. *Iowa, Agric. Home Econ. Exp. Stn., Ames Proj.* **1973**, *No. J.7322*, 1752.

Klepper, L.; Flesher, D.; Hageman, R. H. *Plant Physiol.* **1971**, *48*, 580–590.

Mitchell, G. A.; Bingham, F. T.; Labanauskas, C. K.; Yermanos, D. M. *Soil Sci. Soc. Am. J.* **1976**, *40*, 64–68.

Munck, L., FAO/IAEA/CST Third Research Co-ordination Meetings, Hahnenklee, Federal Republic of Germany, 1975.

Nelson, O. E.; Mertz, E. T.; Bates, L. S. *Science (Washington, D.C.)* **1965**, *150*, 1469–1470.

Radin, J. W. *Plant Physiol.* **1973**, *51*, 332–336.

Rhodes, D. A.; Mathers, J. C. *J. Sci. Food Agric.* **1974**, *25*, 963–972.

Sikka, K. C.; Johari, R. P. *J. Agric. Food Chem.* **1979**, *27*, 12.

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Fatty Acids of Soybean Seeds Harvested from Plants Exposed to Air Pollutants

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The neutral ester, free, and polar fatty acid fractions, obtained from soybean seeds harvested from field-grown plants exposed to SO_2 or O_3, were analyzed in both qualitative and quantitative terms. Plant exposure to SO_2 or O_3 produced seeds of lower oil:protein ratio, but no significant changes in any of the fatty acids were observed. It is suggested that the soybean pod protects the seed from the pollutant and that SO_2 or O_3 exposure of plants within federal guidelines should not affect oil quality.

Several investigators have examined the consequences of chronic air pollutant exposure on the physiology and biochemistry of the soybean [*Glycine max* (L.) Merr.] plant (Heath, 1980; Ziegler, 1975), but relatively few have examined air pollutant effects on seed quality.

The soybean seed consists of protein (30–46%), lipids (12–24%), carbohydrates (33–37%), and minerals (5%). The exact ratio of these constituents depends on the cultivar and environmental conditions under which the plant is grown. The oils and proteins are the commercially important seed constituents (Smith and Circle, 1972). Generally, there is an inverse relationship between seed oil (lipid) and protein content, and if environmental factors alter the seed oil:protein ratio, changes in other components may also occur.

Frey (1972) reported that seeds from soybean plants grown under 980 μg of O_3/m^3 (1.96 mg/m^3 = 1 ppm of O_3) had 4% less oil and 21% more amino acid (protein) than the seeds from control plants. This O_3 concentration is significantly above the national secondary O_3 standard (235

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