Lysine Content of Protein Increased by Germination of Normal and High-Lysine Sorghums

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Sorghum is deficient in lysine for humans. Normal and high-lysine sorghums were germinated for 1–10 days. Lysine content of germinated normal sorghum increased after 10 days from 2.2 to 3.2 g/16 g of nitrogen. For sprouted high-lysine sorghum, lysine content increased from 3.0 to 7.8 g/16 g of nitrogen after 7 days. A large increase in albumin (rich in lysine) and a large decrease in kafirin and cross-linked kafirin (both low in lysine) accompanied sprouting. The percent protein in germinated sorghum is greater than in the initial grain as a result of dry matter loss in the grain during germination, but the absolute amount of protein per kernel is not increased.

Normal sorghum, like most cereal grains, is deficient in lysine (Deyoe and Shellenberger, 1965). Two floury lines of Ethiopian origin are exceptionally high in lysine at relatively high levels of protein (Singh and Axtell, 1973). Another high-lysine sorghum, P-721 opaque, was produced by chemical mutagen treatment of normal grain (Mohan and Axtell, 1975). Normal sorghum has 2 g of lysine/16 g of nitrogen (conventionally assumed to equal 100 g of protein), and the high-lysine sorghums have around 50% more lysine than normal grains. Although the high-lysine sorghums have significantly higher lysine content than the normal ones, their lysine contents are still considerably below the human requirement (National Academy of Sciences, 1974).

One approach to increase the lysine content of sorghum protein is to make a protein concentrate from normal and high-lysine sorghums by alkaline extraction; the concentrate had 50% more lysine/16 g of nitrogen than the starting grain and accounted for 31–38% of the total sorghum protein (Wu, 1978). Apparently the higher lysine content of the protein concentrate is balanced by the lower lysine content of the bran fraction, and the alkali extracted more lysine-rich fractions into the concentrate compared with the starting sorghum (Wu, 1978). Another approach to improve nutritive value of sorghum protein is to germinate the seed (Wang and Fields, 1978). These authors reported that the relative nutritive value of germinated sorghum was substantially improved; however, only 10–35% of the seeds germinated. The low germination levels found by these authors may be a result of artificial drying of the grain after harvesting, the age of the grain, or the conditions used for germination. Tsai et al. (1975) earlier showed that germination increased the lysine and tryptophan levels of corn. Sorghum is germinated extensively in South Africa and serves as a source of malt amylase for production of native beer (Novellie, 1966).

In this study, normal and high-lysine sorghums were germinated for different time intervals, and protein, fat, ash, and starch contents of the sprouted sorghums were determined. The protein from sprouted sorghums was fractionated into different solubility classes, and essential amino acid compositions of the sprouted sorghums and protein fractions were determined.

EXPERIMENTAL SECTION

Sorghum. WAC 694 is a normal sorghum from Texas, and P-721 opaque is a high-lysine sorghum from Indiana. Each sorghum was soaked with about 3 volumes of distilled water overnight, with two changes of water during the day to remove dirt and extra husk. The wet grain was then soaked in 1–2 volumes of 0.2% formaldehyde solution for 40 min to retard mold growth during germination, similar to the method of Fleming et al. (1961). These workers found that the amount of formaldehyde retained in wheat malt was as little as 0.003 ppm and would probably be insignificant so far as human consumption of bread is concerned. Hurd (1920) showed that formaldehyde was absorbed very slowly by wheat and that it was dissipated gradually when wheat was stored moist. The low level of formaldehyde and the short contact time used here for sorghum are designed to minimize any possible deleterious effect. The soaked grain was then washed with distilled water several times and soaked in water for 20 min to remove residual formaldehyde. The wet sorghum was then spread out thinly on Whatman filter papers saturated with distilled water in a container with plenty of air space, and the container was sealed with plastic film and masking tape. For preliminary experiment on a small scale, the grain was allowed to germinate in constant-temperature rooms at 20 and 25 °C in the dark and at average temperature of 23 °C in a room with daylight for 1–9 days, using a different container for each condition. One hundred kernels of sprouted sorghum were dried at 105 °C to constant weight and then ground twice in a Wiley mill for nitrogen and moisture determinations. Any moldy grain was discarded.

For most of the experiments described in this paper, the normal sorghum was germinated at an average temperature of 26 °C in a room with daylight, and the high-lysine sorghum was germinated at an average temperature of 22 °C. After 1 day, 66% of the grain sprouted; and after longer periods, 74–86% of the grain sprouted. No mold was observed for normal sorghum after 6 days of germination, but about 25% of the grains were moldy after 10 days. A small number of high-lysine sorghum grains contained mold after 7 days of germination, and about 50% of the grains showed mold after 9 days. The moldy grains were discarded. The sprouted and unsprouted grains were freeze-dried separately and ground twice in a hammer mill with a screen containing $^1/_{16}$ in. diameter holes. The higher mold growth on high-lysine sorghum may be a result of less thorough cleaning of the grain when received compared with the normal sorghum. The mold problem in general seems to require a good mold inhibitor as formaldehyde to treat the grain before germination.

Protein Extraction. Each sample (10 g) was put in a steel cup with 100 mL of solvent and blended for 5 min in a Waring Blendor. The schematic diagram for extracting protein from ground sorghum is shown in Figure 1. Each sample after blending was centrifuged at 10400*g*

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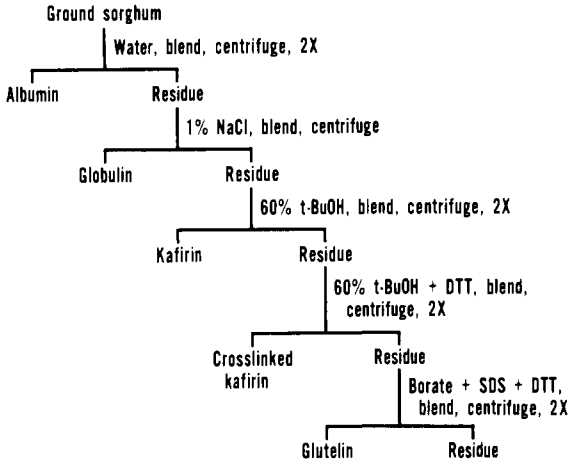

Figure 1. Schematic diagram for extracting protein from ground sorghum. *t*-BuOH is tertiary butanol, DTT is dithiothreitol, SDS is sodium dodecyl sulfate, and cross-linked kafirin is used interchangeably with alcohol-soluble reduced glutelin. 2× means the material is extracted twice with the particular solvent and the supernatants from the two extractions are combined.

for 10 min. The solvents used were water, 1% NaCl, 60% *tert*-butyl alcohol (*t*-BuOH), 60% *t*-BuOH + 0.05% dithiothreitol (DTT) and borate (0.0125 M borax + 0.043 N sodium hydroxide + 0.425 M sodium chloride) + 0.5% sodium dodecyl sulfate ($NaDodSO_4$) + 0.05% DTT buffer at pH 10 described by Landry and Moureaux (1970). This series of solvents extracted albumin, globulin, kafirin, cross-linked kafirin and glutelin, respectively, as outlined in Figure 1. The combined 60% *t*-BuOH + DTT extracts contained alcohol-soluble reduced glutelin or cross-linked kafirin. The former name was originally used by most authors, but the latter name was used by Guiragossian et al. (1978). Paulis and Wall (1979) found that the polyacrylamide gel electrophoresis (PAGE) and $NaDodSO_4$–PAGE patterns of kafirin and alcohol-soluble reduced glutelin of the same sorghum variety appeared qualitatively identical. It will be seen later in this paper that amino acid composition of cross-linked kafirin is almost identical with kafirin, but it differs considerably from glutelin. Consequently, cross-linked kafirin and alcohol-soluble reduced glutelin will be used interchangeably in this paper.

Composition. Protein, fat, and ash were determined by microKjeldahl, petroleum ether extraction and heating to 600 °C as described by procedure numbers 46–13, 30–26, and 08–16 of AACC Approved Methods (1971), respectively. Protein is calculated from N × 6.25 and includes also any free amino acids and other nitrogen compounds. Moisture was determined by heating samples at 105 °C to constant weight, and starch was measured by a polarimetric method (Garcia and Wolf, 1972). Protein was determined in triplicate and fat, moisture, and starch determinations were in duplicate.

For amino acid analysis, each sample was hydrolyzed for 24 h by refluxing in 6 N hydrochloric acid. The hydrolyzed sample was evaporated to dryness in a rotoevaporator, and the residue was dissolved in pH 2.2 citrate buffer. A portion of the acid hydrolyzate was analyzed in a Beckman Spinco Model 121 amino acid analyzer, and the data were computed automatically by the method of Cavins and Friedman (1968).

RESULTS AND DISCUSSION

Dry Weight and Absolute Amount of Protein. Weight and protein changes of 100 kernels of normal and high-lysine sorghums during germination at constant temperatures of 20 and 25 °C in the dark and at average temperature of 23 °C in a room with daylight are listed in Table I. For normal sorghum, dry weight loss does not differ greatly at different temperatures and light conditions for the first 6 days, and there is no significant change in the absolute amount of protein in the kernel. At 9 days of germination for normal sorghum, there is 10% weight loss and no change in absolute amount of protein in a room with daylight, but a higher weight loss and some loss in absolute amount of protein does occur in the dark. The loss is higher for both weight and protein at 25 °C than at 20 °C. For high-lysine sorghum, there is a small weight loss but no change in absolute amount of protein at 3 days of germination (Table I). Except at 20 °C, weight loss is more than 10% at 5 days of germination and there is some loss in protein. A higher loss in weight and protein is evident at 8 days of germination, and the loss is less at 20 °C than at 23 and 25 °C independent of light. We found that the best condition for germinating normal sorghum was in a room with daylight; therefore, a room with daylight at an average temperature of 26 °C was used for subsequent work. Since a lower temperature is best for high-lysine sorghum, an average temperature of 22 °C was used for the rest of the experiments.

Table I. Weight and Protein Changes of 100 Kernels of Normal (n) and High Lysine (hl) Sorghums During Germination (Dry Basis)

days sprouted	temp, °C	light	weight loss, %		protein change, %	
			n	hl	n	hl
1	20	no	4			
	25	no	6			
2	20	no	5			
	25	no	5			
3	20	no	7	3	1	0
	25	no	1	5	0	−1
5	23	daylight		15		−6
	20	no		11		0
	25	no		15		−5
6	23	daylight	8		−2	
	20	no	6		−1	
	25	no	8		1	
8	23	daylight		21		−8
	20	no		15		−5
	25	no		24		−15
9	23	daylight	10		1	
	20	no	18		−5	
	25	no	23		−9	

Table II. Compositions of Normal (n) and High-Lysine (hl) Sorghums at Different Stages of Germination in Daylight (Percent Dry Basis)

treatment	protein, N × 6.25		fat		ash		starch
	n	hl	n	hl	n	hl	n
none	10.1	15.9	3.5	4.9	1.7	1.8	75
soaked 1 day	9.8	16.0	3.7	5.5	1.2	1.5	75
1-day sprouted	10.1		3.7		1.5		72
2-day sprouted	10.2		3.6		1.5		73
3-day sprouted	10.3	16.9	3.5	5.8	1.5	1.8	74
6-day sprouted	11.1		4.1		1.7		66
7-day sprouted		18.6		6.5		2.1	
9-day sprouted		21.3		7.0		2.3	
10-day sprouted	11.9		3.3		1.8		54
6-day incubation, unsprouted	10.3						
7-day incubation, unsprouted		17.3					

Composition. The changes in compositions of normal sorghum at different stages of germination are summarized in Table II. In the first 3 days, the normal sorghum did not differ much in protein, fat, ash, and starch. The 6-day

Table III. Protein Distribution of Normal (n) and High-Lysine (hl) Sorghums as Germination Proceeds in Daylight

	% total protein of fraction at day of germination								
	0		1	3		6	7	9	10
fraction	n	hl	n	n	hl	n	hl	hl	n
albumin	15	18	14	22	32	36	41	48	43
globulin	1	3	2	2	3	3	3	4	3
kafirin	17	19	19	16	16	12	10	5	10
cross-linked kafirin	31	22	32	22	13	12	6	2	6
glutelin	25	23	23	26	24	23	27	23	19
residue	10	9	13	16	11	12	8	10	14
total	99	94	103	104	99	98	95	92	95

sprouted sorghum had higher protein and lower starch contents compared with the starting grain, and a further increase in protein and decrease in starch contents were observed for the 10-day sprouted sorghum. Fat and ash contents did not show any trend as germination proceeded. The 6-day unsprouted grain had similar protein content as the starting material.

For the high-lysine sorghum, the 3-day sprouted grain began to show significant increases in protein and fat contents compared with starting material (Table II), and the increase in protein and fat contents continued for the 7-day and 9-day sprouted sorghums. The 7-day unsprouted grain also had considerably higher protein content than the starting material, although the increase in protein content for the unsprouted sorghum was only about half of the increase compared with the sprouted material. A small increase in ash content was observed for the 7-day and 9-day sprouted sorghum, but this increase may not be significant.

Protein Fractions. Normal sorghum at various stages of sprouting was fractionated by a series of solvents (Figure 1) into different solubility classes as summarized in Table III. The 1-day sprouted grain and the unsprouted grain were almost identical in the relative amounts of each protein fraction. The 3-day sprouted sorghum had higher albumin and lower cross-linked kafirin compared with the starting material. During further germination, the albumin fraction continued to increase and cross-linked kafirin continued to decrease for the 6-day and 10-day sprouted grain; in addition, kafirin fraction also decreased as compared with unsprouted sorghum. There was a small increase in globulin fraction but no definite trend for glutelin and residue fractions as sprouting proceeded.

For high-lysine sorghum, an increase in albumin and a decrease in kafirin and cross-linked kafirin accompanied sprouting (Table III). However, the globulin, glutelin, and residue fractions did not change. After 9 days of sprouting, about half of the protein was albumin; but kafirin and cross-linked kafirin almost disappeared.

Amino Acid Composition. The essential amino acid composition of normal sorghum at different stages of sprouting is listed in Table IV, together with the National Academy of Sciences (1974) pattern of high-quality protein for human consumption. This National Academy of Sciences pattern includes an upward adjustment of 30% for individual variability. The grain that was soaked 1 day had the same essential amino acid composition as the starting material. For the first 3 days of sprouting, a small increase occurred in isoleucine, in lysine, and in methionine + cystine. For 6-day and 10-day sprouted grain, a further increase in lysine and a decrease in leucine were observed. The 6-day sprouted sorghum had higher isoleucine and methionine + cystine, and the 10-day sprouted grain had lower phenylalanine + tyrosine but higher valine than the starting material. The 6-day unsprouted grain had higher isoleucine, leucine, and lysine but lower methionine + cystine compared with the starting sorghum. The 6-day sprouted sorghum met or exceeded the National Academy of Sciences pattern for all essential amino acids except lysine, whereas untreated sorghum was deficient in lysine. A larger increase of lysine content from 2.2 to 3.1 g/16 g of nitrogen was observed for the 6-day sprouted grain. This increase in lysine may improve the PER substantially, because lysine is the first limiting amino acid in sorghum. Tryptophan was not analyzed here. Wang and Fields (1978) found that available lysine increased 2.2 times, methionine 1.8 times, and tryptophan 5.7 times for sorghum seeds germinated 3–4 days at 30 °C over the same amino acid values of the control sample based on microbiological assay.

For high-lysine sorghums at various stages of sprouting, the essential amino acid composition is shown in Table IV and compared with the National Academy of Sciences pattern. The untreated grain had 3.0 g of lysine/16 g of nitrogen, compared with 2.2 g for untreated normal sorghum and 3.1 g for 6-day sprouted normal grain. In spite of this much higher content of lysine for high-lysine sorghum compared with normal grain, the former was still low in lysine compared with the National Academy of Sciences pattern. The grain that was soaked 1 day had practically the same composition as the starting material. The most striking change in amino acid composition as sprouting proceeded was the large increase in lysine content. The 3-day sprouted sorghum also had higher methionine + cystine and valine, and the 7-day and 9-day sprouted sorghums also had lower leucine and phenylalanine + tyrosine but higher valine compared with the starting grain. The 7-day incubated, unsprouted sorghum had slightly higher lysine and methionine + cystine than the starting material, but the change may not be significant. The sprouted sorghums (3, 7, and 9 days) met or

Table IV. Essential Amino Acid Composition of Normal (n) and High-Lysine (hl) Sorghums as Germination in Daylight Proceeds (g/16 g of Nitrogen Recovered)

					days sprouted							days unsprouted		
	sorghum		soaked 1 day		1	2	3		6	7	9	10	6	7	NAS[a]
amino acid	n	hl	n	hl	n	n	n	hl	n	hl	hl	n	n	hl	(1974)
Ile	3.9	4.2	3.8	4.4	4.2	4.6	4.5	4.6	4.6	4.6	4.4	4.2	4.5	4.6	4.2
Leu	13.2	13.7	13.0	14.2	13.4	13.9	13.3	13.0	11.9	9.8	8.2	8.8	15.1	14.5	7.0
Lys	2.2	3.0	2.1	3.3	2.3	2.5	2.7	5.1	3.1	7.8	6.7	3.2	2.6	3.4	5.1
Met + Cys_2	2.6	2.6	2.8	2.4	3.8	3.6	3.6	3.2	3.6	3.7	2.8	2.5	1.7	3.0	2.6
Phe + Tyr	10.6	9.8	10.0	10.4	10.3	10.7	10.5	10.1	10.1	9.0	7.6	7.3	10.7	10.3	7.3
Thr	3.4	3.4	3.7	3.5	3.5	3.7	3.5	3.5	3.7	3.7	3.9	3.7	3.3	3.4	3.5
Val	5.4	4.9	5.5	5.1	5.5	6.0	5.9	5.5	5.9	6.1	5.3	6.1	5.0	5.2	4.8

[a] National Academy of Sciences pattern of high-quality protein for human consumption. This pattern includes an upward adjustment of 30% for individual variability.

Table V. Essential Amino Acid Composition of Protein Fractions from 3-day Sprouted (in Daylight) Normal (n) and High-Lysine (hl) Sorghums (g/16 g of Nitrogen Recovered)

	albumin		globulin		kafirin		cross-linked kafirin		glutelin	
amino acid	n	hl	n	hl	n	hl	n	hl	n	hl
Ile	3.4	4.2	4.0	3.8	4.4	4.7	4.8	4.8	4.6	4.8
Leu	6.4	8.6	7.1	6.8	17.8	19.2	19.6	20.4	10.9	11.5
Lys	3.9	8.4	2.7	5.8	0.3	0.4	0.0	0.1	4.1	5.3
Met + Cys_2	1.8	2.5	4.1	3.6	2.5	1.3	1.9	1.8	4.1	3.6
Phe + Tyr	6.4	8.4	10.3	7.7	11.3	11.2	13.2	12.7	9.8	10.1
Thr	3.4	3.7	3.6	4.0	2.8	2.7	2.6	2.6	4.3	4.2
Val	5.2	6.1	5.4	4.6	5.5	4.7	5.1	4.5	7.1	6.1

exceeded the National Academy of Sciences pattern and appear to be good sources of protein nutritionally.

The essential amino acid composition of protein fractions from 3-day sprouted normal sorghum is listed in Table V. Kafirin and cross-linked kafirin have high levels of leucine but little or no lysine. Albumin and glutelin had higher lysine, whereas globulin and glutelin have higher methionine + cystine than other fractions. As sprouting proceeded, kafirin and cross-linked kafirin with little or no lysine were hydrolyzed and replaced by albumin with higher lysine (Tables III and V). The increase in lysine content of sprouted sorghum in Table IV can be qualitatively accounted for by this replacement of kafirin and cross-linked kafirin by albumin. However, this simple replacement of kafirins by albumin does not rule out more complicated explanation and does not imply that albumin fraction has constant composition during germination.

For high-lysine sorghum, the essential amino acid composition of protein fractions from 3-day sprouted grain is also shown in Table V. Kafirin and cross-linked kafirin both have high levels of leucine and little or no lysine. There is no large difference between kafirin and cross-linked kafirin in amino acid composition, and no difference in cross-linked kafirin from normal and high-lysine grains. In general, there is no large variation in amino acid composition of the same solubility fraction from normal and high-lysine sorghums, except that in the latter albumin has higher isoleucine, leucine, lysine, methionine + cystine, valine, and phenylalanine + tyrosine; globulin has higher lysine but lower methionine + cystine, phenylalanine + tyrosine, and valine; kafirin has lower methionine + cystine; and glutelin has higher lysine but lower methionine + cystine and valine. The increasing lysine content with sprouting of high-lysine sorghum can be qualitatively accounted for by the replacement of kafirin and cross-linked kafirin (both low in lysine) by albumin (high in lysine) in Tables III and V. Again, the data do not rule out a more complicated explanation.

Besides the change in essential amino acids in Table IV during germination, the normal sorghum after 10 days of germination had less arginine, glutamic, proline, and alanine but higher aspartic and glycine, and the high-lysine grain had less glutamic, proline, and alanine but higher aspartic and glycine compared with the starting material (not shown in table).

CONCLUSION

The significant increase in lysine contents (expressed as grams per 16 g of nitrogen or per 100 seeds) of normal and high-lysine sorghums during sprouting appears to indicate a simple method to improve the apparent nutritive value of sorghum protein. The real nutritive value of sorghum protein after sprouting will have to await human or rat feeding studies. The sprouting of high-lysine sorghum is even more favorable than normal grain, because larger increases in lysine content (expressed as a percentage basis or absolute amount) were observed. The high percentage of sprouting grain may make the removal of unsprouted sorghum unnecessary while still realizing a large increase in lysine content of the sorghum. The sprouted sorghum can be used as vegetable, salad, or dried and ground to flour in more traditional food uses.

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