Proximate Composition and Proteins of Three Grain Sorghum Hybrids and Their Dry-Mill Fractions

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In a study of the essential constituents of three grain sorghums, special emphasis was placed on their protein fractions. Sorghum was milled in a Buhler mill to obtain bran, shorts, and flour fractions. Each fraction was extracted successively with water, 1% sodium chloride, and 60% alcohol (*t*-butyl alcohol at room temperature or ethanol at 60° C.). The three sorghums had similar chemical compositions and milling properties. Also similar were solubility, electrophoretic patterns, and amino acid content of the protein.

Introduction of hybrid grain sorghum has greatly increased production of this crop in the United States and promises to do so throughout the world. In the United States the yield has risen from 90 million bushels in 1952 to an estimated billion bushels in 1968. A number of reports record the amino acid composition of grain sorghums (Deyoe and Shellenberger, 1965; Virupaksha and Sastry, 1968), but relatively little is known about the proteins of this cereal. Sastry and Virupaksha (1967) have described the physical properties of the protein of an Indian variety of grain sorghum.

We studied three American hybrid grain sorghums with special emphasis on their protein fractions. Proteins from various mill fractions were compared since any differences are important if sorghum is to be used as human food. Special attention was given to the endosperm fraction.

The water, salt, and alcohol-soluble proteins were extracted from each mill fraction. Amino acid compositions and electrophoretic patterns were obtained. Special attention was given the alcohol solubles because they represent 37% of the total endosperm protein. Water and salt solubles together are only 6% of the total.

MATERIALS AND METHODS

Grain. The sorghum hybrids were OK612, RS626, and TE77. They were grown side-by-side in the same field in 1967 at South Plains Research and Extension Center, Lubbock Texas. RS626 is a head smut resistant RS610 type. It is a medium early maturing hybrid commonly grown under dryland conditions. TE77 is a common, full-season hybrid which gives a high yield under irrigated conditions in West Texas. OK612 is a hetero-yellow endosperm hybrid. The pollinator has yellow endosperm; the grain, a reddish color due to the female parent. This hybrid is particularly interesting because a true yellow endosperm hybrid needs to be developed. Table I lists the analyses of these grains.

Starch content of the samples was determined polarimetrically (Earle and Milner, 1944). Protein ($N \times 6.25$) was measured by semimicro-Kjeldahl. Crude fiber and ash were estimated by the official AOAC methods (1965). No major differences were observed in the chemical composition of the three hybrid sorghum grains.

Milling. Sorghum was milled in a Buhler mill. Bran, shorts, and flour fractions were obtained. The bran contained most of the germ and the hull, but here it will be referred to as the bran fraction. Shorts are composed of endosperm contaminated with bran.

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Table I. Composition of Whole Grain Sorghums

Component	% of Total Weight (Dry Basis) OK612	RS626	TE77
Protein	11.6	11.5	11.7
Starch	75.9	76.3	75.9
Fat[a]	3.3	3.1	3.4
Fiber	1.9	1.8	...
Ash	1.3	1.2	...

[a] Hexane soluble material.

Isolation of Protein. To isolate protein, lipid was first removed from the mill fractions with *n*-butyl alcohol (Jones and Dimler, 1962). The defatted fractions were extracted at room temperature with three times their weight of distilled water. This step was done in a Waring Blendor for 1 minute at high speed and 3 minutes at low speed. The dispersion was centrifuged at 10,400 G for 10 minutes. The residue was similarly extracted successively with 1% sodium chloride, distilled water (to remove salt), and twice with 60% *t*-butyl alcohol. In determining alcoholic concentration, the water in the residue was allowed for. The alcohol extracts were combined. The second water wash was discarded since it contains only a trace of protein. The water and salt extracts were dialyzed against distilled water and freeze-dried. The *t*-butyl alcohol solubles were freeze-dried without dialysis.

For comparative purposes, in some experiments 60% ethanol at 60° C. replaced the room temperature *t*-butyl alcohol. Sixty per cent *t*-butyl alcohol at 60° C. was also tried. The ethanol solution had to be dialyzed against water before it could be freeze-dried. The ethanol-soluble protein precipitated during dialysis. It was difficult to redissolve the precipitate.

Electrophoresis. Electrophoresis was carried out in 5% polyacrylamide gel. The buffer used was aluminum lactate–lactic acid at pH 3.1 (Jones *et al.*, 1959) containing 8*M* urea. The apparatus was described by Cluskey (1964). The amount of catalyst used had to be carefully controlled to ensure proper gelling of the acrylamide. Seventeen and one-half grams of Cyanogum 41, 168 grams of urea, 0.86 gram of aluminum lactate, and 1.31 ml. of lactic acid were dissolved in water to give a total volume of 350 ml. Then 1.31 ml. of *N,N,N',N'*-tetramethyl ethylenediamine followed by 0.65 gram of ammonium persulfate were added. The solution was quickly poured into the mold. It is not necessary to protect the solution from air. The gel was prerun overnight to a constant current to remove excess catalyst. The protein sample (about 4%) was added to slots formed in the gel and run at 400 V.

Table II. Analysis of Dry-Milled Fractions[a] of Grain Sorghums

Fraction	Protein, %	Fat, %	Fiber, %	Ash, %	Weight, %
Bran	14.6	13.3	7.5	4.2	
Shorts	17.0	1.0	1.4	0.4	
Flour	9.8	1.3	0.4	0.6	
Distribution of Constituents in Total Grain					
Bran	22	72	74	62	18
Shorts	20	4	11	5	14
Flour	58	29	15	34	69

[a] Average of three hybrids.

Table III. Amino Acids of Sorghum Mill Fractions

Amino Acid	G./16 G. Nitrogen			
	Grain	Flour	Shorts	Bran
Lysine	1.8	1.7	0.8	3.8
Histidine	2.1	2.1	1.7	2.5
Ammonia	3.3	3.8	3.1	2.4
Arginine	3.2	2.8	2.1	6.2
Aspartic	7.0	7.4	7.0	8.7
Threonine	3.5	3.5	3.5	4.2
Serine	4.6	4.9	4.8	4.9
Glutamic	24.9	24.5	27.1	16.4
Proline	9.0	8.9	10.3	5.1
Glycine	3.2	3.2	2.5	5.3
Alanine	9.9	10.5	11.0	7.2
½ Cystine	0.7	0.4	0.7	1.2
Valine	4.9	5.2	4.9	4.9
Methionine	1.3	1.2	1.1	1.4
Isoleucine	3.9	4.5	4.8	3.8
Leucine	14.5	16.3	16.8	9.1
Tyrosine	4.6	5.0	4.9	3.7
Phenylalanine	5.3	5.4	5.6	4.2

Table IV. Solvent Extraction of Mill Fractions

Fraction	% of Total Nitrogen Extracted					
			60% at Room Temperature		60% at 60° C.	
	H_2O	1% NaCl	*t*-BuOH	EtOH	*t*-BuOH	EtOH
Flour[a]	3	3	37	3	35	40
Short[b]	0.5	0.8	44	...	...	...
Bran[b]	12	10	14	...	...	...

[a] Average of three hybrids. [b] Average of two hybrids.

Amino Acid Analysis. Samples for amino acid analyses were hydrolyzed for 24 hours in refluxing constant boiling HCl. Sample-to-acid ratio was approximately 1 mg. to 2 ml. Hydrolysates were evaporated to dryness, and the residue was dissolved in pH 2.2 citrate buffer for analysis.

The 3-hour analytical procedure of Benson and Patterson (1965) was used on a Beckman Spinco Model 120 amino acid analyzer. Intergration of peaks was accomplished electronically with an Infotronics-Integrator while the amino acid data were automatically computed (Cavins and Friedman, 1968).

RESULTS AND DISCUSSION

The three hybrid sorghums have almost identical chemical analysis and milling characteristics. Protein solubility of the varieties, as well as the amino acid composition and electrophoretic patterns of the fractions, are the same within experimental error. Therefore, most of the results reported are average values of the three sorghums.

Given in Table II is an analysis of fractions of sorghum grain produced by dry milling. The high fat, fiber, and ash content of the bran indicates efficient milling. The percentage distribution of components in each fraction also is listed in Table II. The flour, which was the most extensively studied fraction, contained 69% of the total weight and 58% of the total protein of the grain.

The amino acid content of whole grain and mill fractions is found in Table III. The grain and flour were very similar in amino acid composition. The shorts contained less lysine, histidine, arginine, and glycine than did the whole grain or flour. The bran contains about twice as much lysine, arginine, cystine, and glycine and less proline, leucine, and glutamic acid than do the other samples.

Amino acid analyses have been made on kernels of a number of varieties and hybrids of grain sorghums (Deyoe and Shellenberger, 1965; Virupaksha and Sastry, 1968). Except for tyrosine, the amino acid content of our sorghum grains falls within the range found by others. Our sorghums contain twice as much tyrosine as reported in other varieties. The lower tyrosine content was probably due to destruction of this amino acid during hydrolysis.

Table IV lists the amount of nitrogen extracted with each

Table V. Amino Acids of Sorghum Extracts

Amino Acid	G./16 G. Nitrogen					
	Flour				*t*-Butanol soluble	
	H_2O Soluble	NaCl Soluble	*t*-BuOH Soluble	Residue	Shorts	Bran
Lysine	6.3	4.7	0.1	2.1	0.0	1.1
Histidine	2.9	2.5	0.9	2.0	0.9	1.2
Ammonia	1.4	1.5	3.5	3.2	3.4	4.3
Arginine	6.9	10.8	1.0	3.6	1.4	1.9
Aspartic	10.4	7.9	6.5	5.6	6.2	6.5
Threonine	6.0	4.6	2.6	3.5	2.4	3.0
Serine	4.8	5.7	4.1	4.4	3.7	4.1
Glutamic	14.2	13.7	30.0	23.3	31.8	25.4
Proline	6.0	4.5	10.0	10.9	11.0	8.4
Glycine	7.0	6.4	1.1	2.4	0.8	2.3
Alanine	7.4	6.0	12.4	9.1	10.3	9.6
½ Cystine	1.4	2.9	0.4	1.2	0.0	0.0
Valine	6.0	5.3	5.0	5.1	5.1	4.9
Methionine	2.0	1.4	1.0	1.4	1.4	0.6
Isoleucine	4.3	3.5	4.8	4.3	4.2	4.0
Leucine	7.5	6.4	19.2	14.9	20.6	16.2
Tyrosine	3.7	3.3	5.5	5.1	5.8	4.9
Phenylalanine	3.9	4.1	6.4	5.5	6.4	5.4

Table VI. Protein Analysis of Solids in Extract from Different Milling Fractions

Soluble	% Protein ($N \times 6.25$) Flour	Shorts	Bran
Water			
OK612	12	5	52
RS626	25	2	61
TE77	9	...	...
NaCl			
OK612	23	5	54
RS626	20	3	61
TE77	13	...	...
t-BuOH			
OK612	95	91	43
RS626	90	91	44
TE77	90	...	...

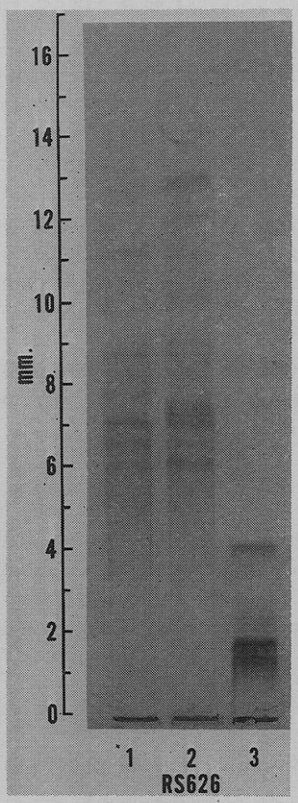

Figure 1. Electrophoretic patterns of proteins from grain sorghum flour (RS626)

Aluminum lactate–lactic acid buffer, 8*M* urea, pH 3.1, 400 V, 2½ hours. 1. Water solubles; 2. sodium chloride solubles; and 3. *t*-butyl alcohol solubles

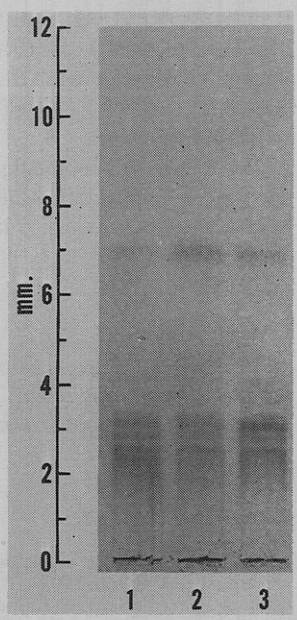

Figure 2. Electrophoretic patterns of proteins from sorghum flours

Aluminum lactate–lactic acid buffer, 8*M* urea, pH 3.1, 400 V, 2½ hours, *t*-butyl alcohol solubles. 1. OK612; 2. RS626; and 3. TE77

solvent. After extraction with water and with 1% sodium chloride, the prolamine can be dissolved with 60% *t*-butyl alcohol at either room temperature or 60° C. If 60% ethanol is used, it must be heated to 60° C. The shorts and bran fractions of TE77 were not extracted.

The amino acid content of the extracts is given in Table V. The *t*-butyl alcohol solubles from flour are relatively low in lysine, arginine, glycine, cystine, histidine, and threonine. They contain more leucine, glutamic acid, proline, and alanine than do the water or salt solubles.

Alcohol solubles from flour and shorts have more than 90% protein (Table VI). Other lyophilized solids have much less nitrogen. The water and salt solubles of the shorts are less than 5% protein. The differences, as shown in Table VI, and the amount of pigment are the only noticeable changes among the hybrids noted in our work.

The alcohol fractions were colored deep red. Sorghum TE77 had the most color and OK612 the least. Among the mill fractions the bran had the most pigment and the flour the least. The alcohol solubles can be decolorized by passing a *t*-butyl alcohol solution of the protein over a LH-20 Sephadex column. Two pigments separated, one red and one yellow. The red pigment, but not the yellow, can be removed by adding decolorizing carbon to a *t*-butyl alcohol solution of the protein. Only about 5% of the protein is lost on the carbon. The decolorized product has the same amino acid composition and electrophoretic pattern as the original. Both the *t*-butyl alcohol and ethanol extract contain these colors. Other workers have not reported the presence of pigment in their prolamine fraction since the sorghums they studied were not colored.

The electrophoretic patterns of the three fractions from RS626 flour (Figure 1) show their relative mobilities. The water and salt solubles contain some common bands but others that are different. Components of the alcohol solubles move more slowly than do proteins of the other fractions. This slower mobility is a function of the smaller amount of charged amino acids (lysine, histidine, and arginine) in the alcohol solubles. In each sample there is material at the origin that does not move into the gel. The material that does not move and also the streaking in the alcohol solubles probably relate to the insolubility of the protein. In the other fractions the substance at the origin may be caused by high-nonprotein content.

Figure 2 contains the electrophoretic patterns of the pro-

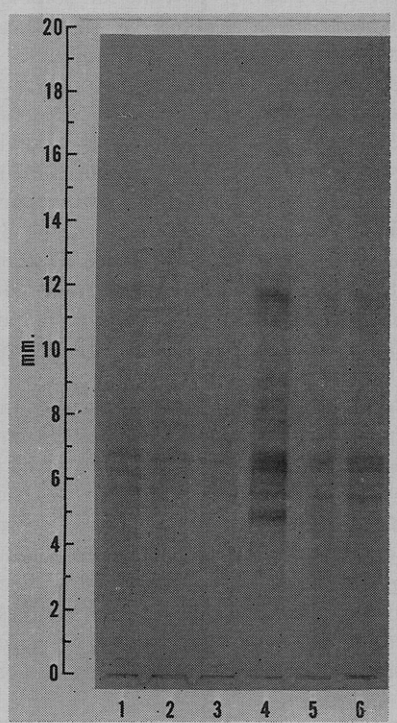

Figure 3. Electrophoretic patterns of proteins from sorghum flours

Aluminum lactate–lactic acid buffer, 8*M* urea, pH 3.1, 400 V, 4 hours. Water solubles; 1. OK612; 2. RS626; and 3. TE77. Sodium chloride solubles: 4. OK612; 5. RS626; and 6. TE77.

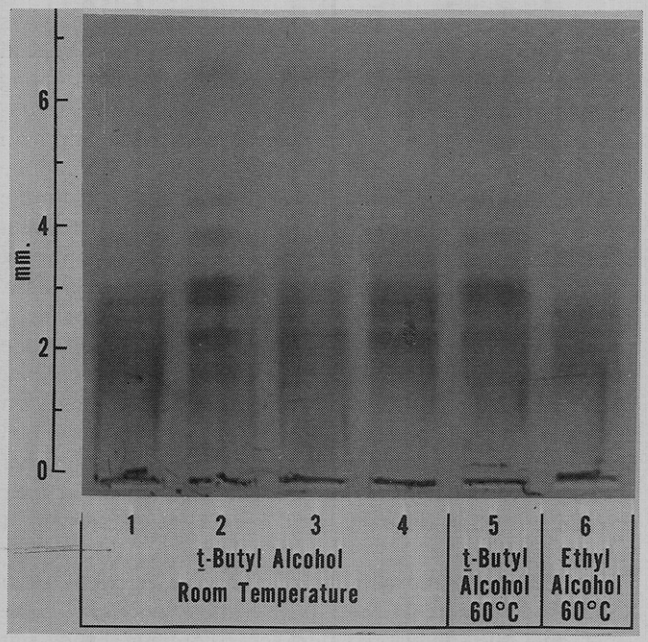

Figure 4. Electrophoretic patterns of proteins from sorghum flours

Aluminum lactate–lactic acid buffer, 8*M* urea, pH 3.1, 400 V, 4 hours. 1. OK612 decolorized; 2. OK612 not decolorized, (3). RS626 decolorized; 4. RS626 not decolorized; 5. RS626; and 6. RS626

lamines from the flour of the three varieties. Electrophoresis time was increased from $2^1/_2$ to 4 hours to give better resolution of the components. Patterns of the three hybrids are identical.

Figure 3 shows the patterns of the water and salt solubles from flour fractions of the three sorghums. The only difference in the three sorghums is that the salt solubles of OK612 has two dark sharp bands at about 4.5 cm. These bands are quite light in the proteins of the other sorghums.

Figure 4 demonstrates the effect of several variables on the electrophoretic patterns of flour proteins. Decolorization did not change the pattern. Extraction with *t*-butyl alcohol at either room temperature or 60° C. produces the same result. There is a slight difference between the patterns obtained with *t*-butyl alcohol and ethanol, especially at the 3-cm. mark. Interestingly enough, a similar difference exists between the butanol and ethanol solubles of proso millet (Jones *et al.*, 1970). The ethanol solubles do contain a component at the 6.5-cm. position, but it is not well defined in the reproduction here.

The electrophoretic patterns of the extracts of the shorts are the same as those from the flour. The patterns from the bran are also essentially the same, but there is so much streaking in the bran that minor differences might not be observed.

Sastry and Virupaksha (1967) obtained electrophoretic patterns of extracts of flour of an Indian sorghum. Using disk electrophoresis and the same buffer we used, they obtained a number of bands in the alcohol solubles. They do not appear to be the same ones we observed from our sorghums. Their water solubles showed only one band and the salt solubles only three.

Great difficulty was encountered in working with the alcohol-soluble fraction; solutions of this protein tend to form a firm gel, and their behavior is not consistent. Sometimes the gel forms a few minutes after extraction from the flour; other times gel formation requires a day; and occasionally, the solution does not gel at all. The alcohol extract of flour or shorts contains 1% protein and of the bran only 0.2%. In either, a firm gel may form. Gelling occurred with protein dissolved in either 60% *t*-butyl alcohol or 60% ethanol. It is faster and takes place more often in *t*-butyl than in ethyl alcohol. Gels also form in protein dissolved in 8*M* urea, dimethyl sulfoxide, or formamide. No gel formed in 6*M* guanidine hydrochloride.

The gel is dissolved by heating and liquefied by vigorous shaking. The solution quickly gels again on cooling or standing. Acrylonitrile or vinyl pyridine was added to freshly extracted protein to block possible disulfide interchange which might cause gelling. This addition had no effect on gel formation. Gels also formed in the presence of 2-mercaptoethanol. Gelling caused some freeze-dried protein to be insoluble. The acrylonitrile or vinyl pyridine treatment did not change solubility. Decolorizing the protein on carbon or LH-20 Sephadex does not prevent gelling.

The tendency to gel differs slightly between proteins from the three sorghum hybrids. The rate of gelling is TE77 > RS626 > OK612. It is noteworthy that the relative rates of gelling of alcohol extracts among these hybrids is related to the amount of pigment in the grain. However, decolorization does not change the rate of gelling. Neither water nor salt-soluble proteins gel.

The nature of the gelling phenomena is not known but it appears to be physical rather than chemical. Gel formation has not been mentioned by other authors.

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