demonstrated with degraded carrageenan (Lin and Hansen, 1970) and has been shown to be associated with a limited range of molecular weight of the carrageenans from 100 000 to 300 000 (Lin, 1971).

We made the observation in this study that carrageenan is subject to a reversible, pressure-dependent dissociation similar to the effect observed for myosin by Josephs and Harrington (1967). Because of this effect, the apparent $\bar{M}_w$ determined by ultracentrifugation would appear to be lower than would be the case for measurements obtained without generation of pressure. Extrapolation to zero speed of the plot in Figure 4 indicates a $\bar{M}_w$ for the undissociated, unheated carrageenan (Seakem 2) of 2.3×10^5.

The number-average molecular weights ($\bar{M}_n$) for several commercial carrageenan types, calculated from intrinsic viscosity data, range from 1.02×10^5 to 2.20×10^5 (Marine Colloids, 1972). Snoeren (1976) obtained by light scattering a value of 7.18×10^5 ($\bar{M}_w$) for the commercial product Genulacta P100 (Kobenhavns Pektinfabrik, Denmark). Smith et al. (1957) estimated the molecular weight of a mixture of high viscosity commercial carrageenans from sedimentation coefficients and intrinsic viscosity data and reported 2.3×10^5 and 3.6×10^5 depending upon the method of relating these parameters. Goring and Young (1955) obtained the value of 12×10^5 ($\bar{M}_w$) for a laboratory sample of unfractionated carrageenan also from sedimentation coefficients and intrinsic viscosity data.

Large variations in the estimates for carrageenan polymer size may be expected because of the heterogeneity of unfractionated carrageenan extracts and because of differences in methods of extraction when heat is involved. However, our observation that carrageenan is an aggregated system which undergoes a pressure-dependent dissociation emphasizes the need to account for this effect when different methods are used in the assessment of the polymer size. Our results show the monomer $\bar{M}_w$ of Seakem 2 carrageenan to be less than 1.0×10^5 and decreasing when heated in a milk salt system.

ACKNOWLEDGMENT

The authors thank R. Townend, USDA, Philadelphia, for assistance with the partial specific volume measurements. One of us (P.M.T.H.) thanks Dairy Research Incorporated, Rosemont, Ill., for a research fellowship.

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Received for review April 12, 1977. Accepted January 17, 1978. Journal Article No. 7:77, Department of Food Science and Nutrition. Supported by Public Health Service Research Grant FD-00117, Office of Research and Training Grants, FDA.

Effects of Dehulling on Tannin Content, Protein Distribution, and Quality of High and Low Tannin Sorghum

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High and low tannin varieties of sorghum grain were subjected to sequential dehulling operations to attempt to remove tannins. Up to 37% of the grains were removed in these procedures. Dehulling resulted in considerable (up to 45%) loss in protein content as well as in the removal of most of the tannins (up to 98%). Amino acid analysis of the dehulled grains showed a progressive decrease in the content of lysine, histidine, and arginine. The solubility distribution pattern of proteins from both varieties at various stages of dehulling showed marked differences. Using the low tannin variety as control, these observations were utilized in assessing the effect of tannins on the solubility characteristics of sorghum proteins. The observed differences are consistent with strong interactions between tannins and the kafirin (prolamin) protein fractions in sorghum.

Sorghum constitutes a major proportion of the world food grain production and is the third largest crop in the United States. A major drawback of sorghum as a food source is the high levels of polyphenols (tannins) associated with certain varieties of sorghum grain. Grain producers and breeders maintain high tannin varieties for their resistance to bird damage, favorable storage quality, desirable weathering, and resistance to preharvest germination. However, the low nutritional quality of high tannin

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0021-8561/78/1426-0679$01.00/0 © 1978 American Chemical Society

Table I. Sequential Dehulling of Normal and High Tannin Sorghum[a]

Sample	Dehulling cycle	Initial charge, lb	Cumulative dehulling time, min	% dehulled	Cumulative dehulling, %
RS626					
Normal	1	58.355	4	11.3	11.3
	2	39.650	16	12.1	23.4
	3	20.185	48	12.6	36.0
BR64	1	64.975	5	12.3	12.3
High Tannin	2	44.560	17	11.9	24.2
	3	23.55	49	12.8	37.0

[a] Data provided by Dr. C. G. Youngs, Prairie Regional Laboratory, Saskatoon, Canada.

Table II. Content and Recovery of Proteins and Tannins in Dehulled Sorghum

Sample	% dehulled	Protein content[a]	Protein recov., %[b]	Tannin content[c]	Tannin remaining, %[b]
BR64	0	9.40	100.0	4.54	100.0
	12.3	8.60	80.2	3.99	77.0
	24.2	8.22	66.3	1.58	26.4
	37.0	8.22	55.0	0.17	2.4
RS626	0	12.81	100.0	0.54	100.0
	11.3	12.57	87.0	0.35	57.5
	23.4	11.94	71.4	0.30	42.5
	36.0	11.29	56.4	0.19	22.5

[a] Expressed as g of protein/100 g of dehulled sample. [b] Based on 100 g of whole sample. [c] Expressed as catechin equivalents/g of sample.

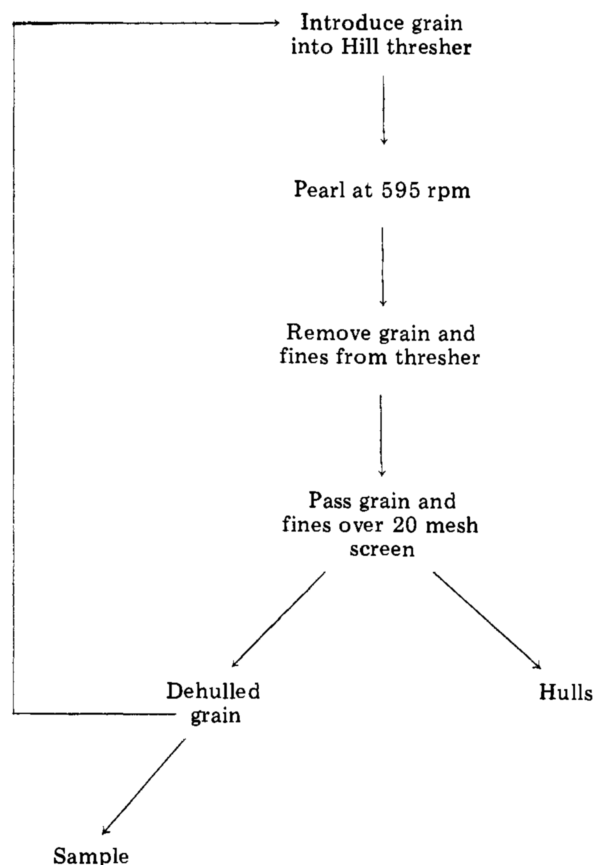
sorghum varieties (Axtell et al., 1975; Jambunathan and Mertz, 1973) has discouraged its use as a primary food source. Numerous efforts have been directed toward coupling the beneficial effects of tannins in sorghum as a field crop with procedures for improving the nutritional quality of the grain. Chemical removal (dehulling) of sorghum tannins with sodium hydroxide (Blessin et al., 1971) removes tannins, which are present primarily in the outer pigmented testa and pericarp (Bate-Smith and Rasper, 1969). Currently, various mechanical dehulling methods are being tested (Reichert and Youngs, 1976). In this report, we describe the effects of mechanical dehulling on the content, quality, and distribution of proteins as well as on the tannin content of normal and high tannin sorghum.

Materials and Methods. A high-tannin, bird-resistant sorghum variety (BR64) and a low tannin line (RS626), grown at the Purdue Agronomy Farm, were from the 1974 harvest. Samples of each variety were subjected to sequential dehulling in a George O. Hill grain thresher at the Prairie Regional Laboratory, Saskatoon, Canada (Scheme I).

A complete description of this thresher has been published (Reichart and Youngs, 1976). It is a commercial abrasive mill which contains thirteen 12 in. diameter Carborundum stones driven at speeds up to 2000 rpm. Grains are fed into the machine through a hopper located at one end and, after action of the stones, are released through an overflow outlet near the other end.

Protein (N × 6.25) was determined by the micro-Kjeldahl procedure. Fractionation of seed proteins was carried out according to the method of Landry and Moureaux (1970) as described in detail earlier (Misra et al., 1975). The individual fractions are obtained by extracting the finely ground sorghum successively with 0.5 M NaCl (fraction I), 70% isopropyl alcohol (fraction II), 70% isopropyl alcohol containing 2-mercaptoethanol (fraction III), borate buffer (pH 10) containing 2-mercaptoethanol (fraction IV), and borate buffer (pH 10) containing 2-mercaptoethanol, and sodium dodecyl sulfate (fraction V). Amino acid analysis was conducted according to Spackman et al. (1958). Tannin content, determined by the vanillin–hydrochloric acid method of Burns (1971) was expressed as catechin equivalents (CE) per gram.

Scheme I



RESULTS AND DISCUSSION

Table I shows the extent of dehulling of both varieties of sorghum after successive passages through the thresher. In each instance, approximately 12% of the grain was removed in the course of each of the three dehulling cycles. On a cumulative basis, 36 and 37% (by weight) was re-

Table III. Amino Acid Composition of Whole and Dehulled Sorghum Grain[a]

	Sample							
	BR64				RS626			
	Percent dehulled				Percent dehulled			
Amino acid	0	12.3	24.2	37.0	0	11.3	23.4	36.0
Lysine	2.2	2.4	1.8	1.3	1.4	1.7	1.2	1.1
Histidine	2.2	2.5	2.2	2.1	2.0	2.1	1.8	2.1
Arginine	4.0	4.2	3.3	2.5	3.3	4.0	2.5	2.7
Aspartic acid	6.1	6.0	5.3	5.3	6.3	6.3	5.4	5.9
Threonine	3.1	3.1	2.5	2.4	2.7	2.7	2.3	2.7
Serine	4.3	4.0	3.6	3.4	3.9	4.0	3.5	4.1
Glutamic acid	19.2	18.3	18.2	18.4	20.4	20.6	20.4	22.6
Proline	8.0	7.0	7.4	7.7	7.7	8.1	7.6	8.0
Glycine	3.4	3.3	2.8	2.3	2.6	2.6	2.1	2.2
Alanine	8.2	7.7	7.5	7.5	8.7	8.8	8.6	9.4
Cystine	2.0	1.3	1.6	0.7	1.3	1.1	0.2	0.7
Valine	4.6	4.5	4.0	3.8	4.3	4.6	4.2	4.7
Methionine	2.2	2.3	2.2	2.1	1.5	1.8	1.4	1.9
Isoleucine	3.6	3.5	3.3	3.3	3.4	3.7	3.5	3.9
Leucine	12.0	11.3	11.3	11.8	12.3	13.1	13.0	14.9
Tyrosine	3.9	3.7	3.8	3.4	3.9	4.1	3.6	4.3
Phenylalanine	4.9	4.4	4.2	4.2	4.7	5.0	4.6	5.3
Ammonia	3.8	3.6	6.2	8.6	2.6	5.4	3.7	3.2

[a] Expressed as g/100 g of protein (N × 6.25).

Table IV. Nitrogen Distribution in Dehulled Sorghums[a]

	Sample							
	BR64				RS626			
	Percent Dehulled				Percent Dehulled			
Protein fraction	0	12.3	24.2	37.0	0	11.3	23.4	36.0
I, albumins and globulins	4.3	3.9	5.0	5.4	13.4	13.6	11.3	5.6
II, kafirin	3.1	2.6	7.1	9.9	8.8	13.1	15.4	17.2
III, crosslinked kafirin (kafirin-like)	11.9	17.4	24.9	33.6	37.2	34.6	30.9	36.3
IV, glutelin-like	17.7	20.4	17.4	12.7	6.6	6.4	6.8	6.2
V, glutelin	63.0	55.7	45.6	38.4	34.0	32.3	35.6	34.7
Total N extracted, %	90.4	89.4	92.7	88.8	84.8	90.1	88.9	88.6

[a] Percent of soluble nitrogen.

moved from low tannin (RS626) and high tannin (BR64) sorghums, respectively.

Table II shows the effect of dehulling on the content of total tannins and on the content and recovery of seed proteins. Progressive dehulling causes a cumulative loss of protein constituents. There was little or no difference between the two varieties in terms of the rate of protein loss. The removal of tannins (in the pertinent case of BR64), however, is marginal subsequent to the first dehulling, though it occurs much more rapidly in subsequent dehulling cycles. Thus, the removal of tannins is inefficient with respect to the extent of dehulling in the early stages of the process. This is probably due to the failure of the mechanized process to "pearl" the grain and represents a combination of chipping and shearing processes aimed at the outer layers of the grain. This is evident upon close examination of dehulled seeds. The shearing process is thus responsible for the removal of significant amounts of the endosperm as well as protein. The combination of these two factors, viz., the low efficiency in the removal of the pigmented testa and pericarp layers, and the unusually high levels of protein loss, make this dehulling process unacceptable as a means of removing tannins from sorghum. The amino acid composition of both varieties of sorghum at various stages of dehulling are shown in Table III. In both instances, progressive dehulling causes a decline in the content of lysine, the first limiting amino acid in sorghum (Axtell et al., 1975). These observations are, furthermore, consistent with a cumulative loss of the embryo, which is rich in both lysine and arginine.

The nitrogen distribution pattern observed in both varieties of sorghum at various stages of dehulling is shown in Table IV. We would expect that, regardless of the tannin content of the testa layers, the two sorghum varieties would not differ significantly in their localization of proteins and protein bodies. Thus, protein distribution changes observed upon dehulling low tannin sorghum (RS626) can serve as controls for observing the effects of tannins on the distribution of proteins in the high tannin variety.

Dehulling of the low tannin variety is accompanied by a decrease in the relative levels of the albumins and globulins, while there is a twofold increase in the relative content of the kafirin fraction (Table IV). The relative levels of the other three protein fractions remain essentially unchanged. The changes observed with BR64 are distinctly different. There is a marginal increase in fraction I, a threefold increase in both fraction II and III, and a sharp decline in the glutelin fractions. In RS626, fractions II and III show only a slight increase.

These alterations in protein distribution patterns are seen in Table V. For the low tannin variety, the contribution of the glutelin proteins to the total protein (as well as to the dehulled grains) does not change significantly, while that of the albumin–globulin fraction declines. There is a marginal enrichment of kafirins at the expense of the albumin–globulin fraction in the later stages of dehulling. Thus, we conclude (1) that the albumin–globulin protein fraction is largely associated with the external portion of the grain (derived from the embryo),

Table V. Content and Recovery of Individual Protein Fractions in Dehulled Sorghums

Protein Fraction	BR64 Percent dehulled	BR64 Percent of[a] soluble protein	BR64 Percent[b] of sample	BR64 Percent[c] Recov.	RS626 Percent dehulled	RS626 Percent of[a] soluble protein	RS626 Percent[b] of sample	RS626 Percent[c] Recovery
Albumins and globulins (I)	0	4.3	0.40	100.0	0	13.4	1.72	100.0
	12.3	3.9	0.34	72.8	11.3	13.6	1.71	88.2
	24.2	5.0	0.41	77.1	23.4	11.3	1.35	60.0
	37.0	5.4	0.44	69.2	36.0	5.6	0.63	23.5
Kafirins (II, III)	0	15.0	1.41	100.0	0	46.0	5.89	100.0
	12.3	20.0	1.72	107.0	11.3	47.7	6.00	90.3
	24.2	32.0	2.63	141.3	23.4	46.3	5.53	71.8
	37.0	43.5	3.58	160.0	36.0	53.5	6.07	66.0
Glutelins (IV, V)	0	80.7	7.59	100.0	0	40.6	5.20	100.0
	12.3	76.1	6.54	75.6	11.3	38.7	4.86	83.0
	24.2	63.0	5.18	51.7	23.4	42.4	5.06	74.5
	37.0	51.1	4.20	34.9	36.0	40.9	4.61	56.8

[a] From Table IV. [b] Based on 100 g of dehulled sample. [c] Based on 100 g of whole sample.

(2) that the glutelins are uniformly distributed in the grain, and (3) that kafirin proteins (derived predominately from protein bodies) are slightly more concentrated in the interior of the seed. Our data on the high tannin sorghum indicates major differences (Table V). In contrast to the behavior of RS626, there is little or no change in the observed content of fraction I proteins, although visual examination of the seeds indicates loss of most of the embryo from which those proteins are predominately derived. Further, we observe a large increase in the kafirin fraction, which when computed on a whole grain basis, provides us with a false 160% recovery of these proteins upon 37% dehulling. Finally, unlike RS626, the contribution of the glutelin proteins to total protein (as well as to the dehulled seeds) declines rapidly. These observations are clearly inconsistent with the conclusions we have drawn concerning the localization of protein constituents in RS626 and can best be explained on the basis of tannin–protein interactions in BR64.

The protein distribution pattern of these two varieties at various stages of dehulling (Table IV) indicate that at maximal dehulling the solubility distribution of proteins from both varieties are quite similar. At this stage, the tannin content of both dehulled samples is negligible (Table II). In the BR64 variety, the large increase in the kafirins may be attributed largely to fraction III, while the sharp decline in the glutelins is mostly derived from the decrease in fraction V proteins. These observations suggest that tannins in sorghum associate predominately and strongly with the kafirins, particularly with the cross-linked kafirins (fraction III), with the result that the kafirin-tannin complexes behave as glutelin proteins with respect to their solubility characteristics. This postulate is consistent with the tannins being aromatic polyphenols and the kafirins highly hydrophobic alcohol-soluble proteins. These two hydrophobic constituents of sorghum are likely to associate strongly in an aqueous environment. Further, the large number of weakly acidic phenolic groups would minimize the solubility of the tannin–kafirin complexes in alcohol (utilized to solubilize kafirins) and enhance their solubility in weakly alkaline detergents (utilized to solubilize the glutelins). Finally, in view of the ability of tannins to bind a large variety of proteins (Jennings et al., 1968), complexes between the tannins and the albumins and globulins may be responsible for the decrease in the observed levels of these proteins in BR64. The preceding arguments are best illustrated in Figure 1. The tannin content of BR64 at various stages of dehulling plays an

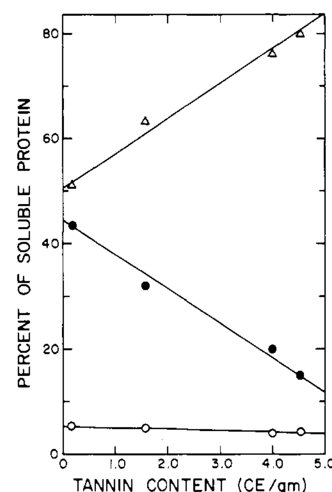


Figure 1. Relative levels of sorghum protein fractions as a function of tannin sorghum (BR64) at various stages of dehulling. Data are from Table V. (O-O-O) Albumins and globulins (fraction I), (Δ-Δ-Δ) glutelins (fractions IV, V), and (●-●-●) kafirins (fractions II, III).

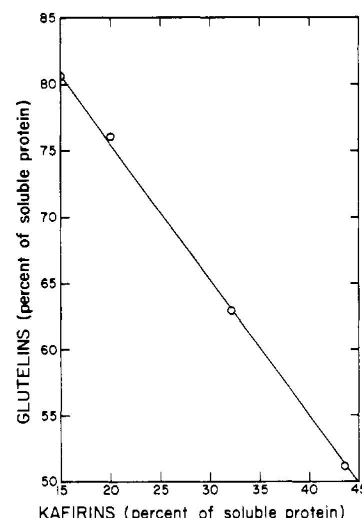


Figure 2. The observed relationship between the relative levels of glutelin and kafirin proteins in high tannin sorghum at various stages of dehulling. Data are from Table V.

important role in determining the solubility of its constituent proteins. No such correlation between tannin

content and protein solubility can be observed in the low tannin variety. Finally, the complementary nature of the kafirin and glutelin distribution is shown in Figure 2. The excellent negative correlation between these two protein constituents in the case of BR64 strongly supports our contention that tannin–kafirin complexes behave as glutelins according to solubility characteristics. In preliminary studies we have compared the ability of the individual sorghum protein fractions to complex with purified tannins prepared from sorghum hulls by the method of Strumeyer and Malin (1975). Fractions II and III showed maximum complex formation leading to precipitation of the complexes from aqueous solution.

ACKNOWLEDGMENT

We are extremely grateful to C. G. Youngs and his associates at the Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada, for their whole-hearted cooperation in the course of this work. We thank Gina H. Y. Lu and M. M. Hassan for technical assistance.

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Received for review March 28, 1977. Accepted February 16, 1978. Journal paper no. 6655 of the Purdue Agricultural Experimental Station. Supported by the U.S. Agency for International Development under a contract titled "Inheritence and Improvement of Protein Quality and Content in *Sorghum bicolor* (L.) Moench".

Some Physicochemical Properties of Peanut Protein Isolates

N. J. Neucere* and E. J. Conkerton

Peanut proteins were separated by ion-exchange chromatography to yield five fractions. These were characterized by immunochemistry and electrophoresis. The bulk of the protein was concentrated in two fractions, representing the conarachin and arachin systems. One of the five protein fractions contained relatively high concentrations of methionine, lysine, and cystine. Studies of solubility in acidic sucrose buffers showed evidence that peanut protein isolates could possibly be utilized in high-protein citric acid-based beverages.

Isolation, characterization, nutritional quality, and functional properties of plant proteins have been the objects of intense studies in recent years. Protein isolates and concentrates from seeds play an important role in the fabrication of modern foods such as milk-like drinks and imitation meats. Soybean proteins, in particular, dominate the market for producing foods such as meat analogues and extenders and protein-rich beverages. Current literature on this subject, however, indicates that more of the other oilseeds and high-protein legumes will be used in diverse staple food products in the future.

In peanuts, the water- and salt-soluble proteins and enzymes have been characterized according to their elution patterns from chromatography on ion-exchange cellulose, and by other physicochemical methods (Cherry et al., 1973; Daussant et al., 1969; Dechary et al., 1961; Dieckert et al., 1962; Neucere, 1969; Thomas and Neucere, 1973). The nutritional quality of protein fractions isolated by density-gradient centrifugation from peanut cotyledons and in heated intact seed was reported by Jacks et al. (1972) and by Neucere et al. (1972). Peanut protein concentrates described by Rhee et al. (1973) have been reported to have potential in baking applications (Khan et al., 1975).

Fractionation of the peanut proteins by chromatography on DEAE–cellulose, reported by Dechary et al. (1961), was accomplished by elution with a linear sodium chloride gradient. The fractions were categorized into four groups: I, II, III, and IV. From the area under each chromatographic peak, it was estimated that the first fraction, I, which was not adsorbed onto the cellulose, comprised about 8.0% of the total proteins soluble in phosphate buffer, pH 7.9, ionic strength 0.03. Groups II and III (the conarachins) and group IV (arachin) comprised about 20, 29, and 43% of the total proteins, respectively. In the present investigation, the objective was to devise a serial elution chromatographic procedure for isolating protein fractions from peanuts that might be useful in large-scale preparations. The isolated fractions were partially characterized by electrophoresis and immunochemistry, tested for solubility in acidic sucrose solutions, and assessed for relative amino acid contents.

EXPERIMENTAL SECTION

Protein Extraction. Ten grams of cotyledons free of testae and axial tissues from Virginia 56R certified peanut seeds were homogenized in a Waring Blendor at medium speed with 30 mL of phosphate buffer, pH 7.9, ionic

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