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In vitro digestibility and amino acid composition of pearl millet (*Pennisetum typhoides*) and other cereals

(pepsin digestibility/protein fractionation/protein quality)

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ABSTRACT **The purpose of this study was to compare *in vitro* digestibility, protein distribution patterns, and amino acid composition of pearl millet with other major cereals. Digestibility values for the pearl millet varieties were higher than that of sorghum and comparable to that of maize. In contrast to sorghum, digestibility of pearl millet and maize did not decrease significantly upon cooking. Protein distribution patterns of uncooked pearl millet and shifts in the different fractions as a result of cooking also resembled that of maize and not sorghum. The amino acid profile of pearl millet is more favorable than that of normal sorghum and normal maize and is comparable to the small grains, wheat, barley, and rice. On the basis of these findings, it appears that pearl millet is a nutritious and well-digested source of calories and proteins for humans.**

Pearl millet (*Pennisetum typhoides*) (Burm.) is one of the major cereal crops of the semiarid regions of Africa and Asia and, because of its drought tolerance and hardiness, it is certainly the mainstay for millions of people in the Sahel. Unlike maize [(*Zea mays* (L.)] and sorghum [*Sorghum bicolor* (L.) Moench], the other important coarse grains of the tropics, very little millet is grown in the developed world. As a result, agricultural research on pearl millet lags behind maize, sorghum, and other food grains. Similar to maize and sorghum, pearl millet is often identified with the poorest of the poor in Africa, where it comprises a significant percentage of the daily food intake prepared in the form of a cooked gruel.

Nutritional studies on sorghum both *in vivo* (1) and *in vitro* (2) have shown that sorghum gruels have a significantly lower protein digestibility compared with rice, wheat, and maize gruels. Nwasike *et al.* (3) and Okoh *et al.* (4) separated whole-grain samples of pearl millet varieties into five fractions by the Landry–Moureaux (5) method and showed that the distribution of proteins in the five fractions was similar to that in maize and not to that in sorghum. Because maize meal when cooked as a porridge has a digestibility that is much higher than that found with sorghum [73% for maize and 47% for sorghum in children as shown by Graham *et al.* (6) and 80% for maize and 60% for sorghum as shown by Hamaker *et al.* (7) using a pepsin method], it was of interest to determine the digestibility of pearl millets.

MATERIALS AND METHODS

The whole-grain samples used were two sorghum varieties, P-721N (normal) and P-721Q (high-lysine opaque), from the 1985 crop at Purdue University Agronomy Farm. The millet samples were Kordofani, a widely cultivated Sudanese variety with a bold yellow grain, grown at Purdue Agronomy Farm in 1985, and a popular food grain variety from the 1985 Niger crop called Zongokolo. Maize samples were not included for the fractionation and *in vitro* pepsin digestion tests inasmuch as adequate data on this cereal were available from previous studies in this laboratory (7, 8). However, for the total amino acid profile assays, several cereals including maize (*Zea mays*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and rice (*Oryzae sativa*) were compared with the same pearl millet and sorghum varieties listed above.

Whole-grain proteins were sequentially extracted into five fractions following the Landry–Moureaux method as described in detail by Misra *et al.* (9). Samples (1 g) of finely ground, defatted, cooked and uncooked flour were suspended in 10 ml of a 0.5 M NaCl solution and stirred for 60 min at 4°C. The mixture was then centrifuged and the extract saved. The residue was treated again with the same amount of saline and stirred for another 30 min. The extraction was repeated for the third time for 30 min. Finally, the residue was extracted with the same volume of water for another 15 min, and this process was repeated once again for 15 min. The five extracts were then combined to give fraction I. The residue was then treated with 10 volumes of 70% isopropyl alcohol at 20°C for three 30-min periods as outlined by Misra *et al.* (9) to give fraction II. The residue was then reserved for isolation of fraction III, etc. Fraction I contains the albumins and globulins, free amino acids and small peptide fragments, and any other saline-soluble compounds. Fraction II contains the prolamins, and fraction III contains prolamin-like proteins that are soluble in alcohol after the disulfide bonds of the protein have been reduced with 2-mercaptoethanol. Fraction IV, which contains proteins soluble in an alkali borate buffer containing 2-mercaptoethanol, has some of the characteristics of glutelin and may be classified as glutelin-like. Fraction V contains the true glutelin, which is a complex, high molecular weight mixture of proteins that can be solubilized only by treatment with a reducing agent and a detergent, $NaDodSO_4$, at alkaline pH. Nitrogen was determined by the micro-Kjeldahl method using the Technicon analyzer.

Both uncooked flour and cooked (1:10 ratio of flour to water in a boiling water bath for 20 min) pearl millet and sorghum samples were digested with pepsin by the method developed by Axtell *et al.* (2) and modified by Mertz *et al.* (10). Thirty-five milliliters of enzyme (pepsin, Sigma P-7000; activity, 120 units/mg of protein) solution (1.5 g of enzyme per liter of 0.1 M KH_2PO_4 buffer, pH 2.0) was added to a 200-mg flour sample, and the resultant mixture was incubated for 2 hr at 37°C in a shaking water bath. The digest was filtered through no. 3 Whatman paper. The residue was resuspended in buffer, centrifuged, dried at 80°C, digested, and colorimetrically assayed for nitrogen content (Technicon Analyzer, AACC 1983). Digestibility was calculated by subtracting residue nitrogen from total nitrogen, dividing by total nitrogen, and multiplying by 100. The pepsin-indigestible residues

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Table 1. Nitrogen distribution in whole seed of pearl millet, sorghum, and maize

Cereal	Variety	% protein in seed	Fraction, % of total protein					
			I	II	III	IV	V	Recovery, %
Millet	52731*	14	22	41	7	9	21	100
Millet	Kordofani	12	27	40	5	5	11	88
Millet	Zongokolo	12	29	38	0	9	16	92
Sorghum	Redlan†	13	10	16	31	4	29	90
Sorghum	P721-N	11	17	15	21	7	31	91
Sorghum	P721-Q	12	24	6	11	7	45	93
Maize	Yellow dent‡	—	20	34	10	10	16	90
Maize	SX52§	11	17	39	10	10	20	96
Maize	Opaque-2§	10	39	12	6	12	32	101

*Ref. 3.
†Ref. 12.
‡Ref. 7; % protein not reported.
§Ref. 8.

were then subjected to the Landry–Moureaux procedure described above.

Amino acid determinations of the various cereals were made after acid hydrolysis (6 M HCl at 110°C for 24 hr) of individual samples on an automated analyzer (Beckman) as described by Spackman *et al.* (11).

RESULTS AND DISCUSSION

The protein distribution patterns in whole kernels of pearl millet, sorghum, and maize varieties are shown in Table 1. The data confirm the finding of Nwasike *et al.* (3) that pearl millet resembles maize in its distribution of proteins, especially with regard to fractions II and III. Fraction II contains the true prolamins, which are soluble in alcohol, and fraction III contains prolamins that are soluble in alcohol only after addition of a reducing agent. The true prolamin fraction (fraction II) in millet accounts for a larger portion of the nitrogen than in sorghum and is comparable to that of maize. The average level of fraction III proteins in normal sorghum (Table 1) is 2–5 times that in normal maize and 6 times that in pearl millet. Similar to both normal maize and normal sorghum, the total alcohol-soluble proteins (fractions II and III combined) in millet account for 38–48% of the total proteins. High-lysine sorghum and maize have lower levels of these two fractions, both of which are low in lysine. Okoh *et al.* (4) also reported a similar large percentage of prolamins in seven early- and late-maturing pearl millet varieties.

Fraction IV protein levels are low and similar in all the three cereals. Millets resemble maize in having about two-thirds of the level of fraction V found in sorghum. The albumin–globulin (fraction I) concentration in pearl millet is higher than in both normal sorghum and normal maize and is comparable to that in high-lysine sorghum.

The ratio of fraction II to fraction III in sorghum is the inverse of that in maize and pearl millet. Hamaker *et al.* (13) suggest that the disulfide cross-linking (primarily in fraction III) may be related to the decrease in digestibility of sorghum on cooking in water. They found that reducing agents added prior to cooking prevent most of the decrease in digestibility. In this process (i.e., chemical reduction), fraction III proteins are converted to polypeptides with the same electrophoresis characteristics as chemically reduced fraction II proteins

Table 2. Effect of cooking on pepsin digestibility of millet, maize, and sorghum

Cereal	Variety	Protein digestibility,* %	
		Uncooked	Cooked
Millet	Kordofani	91 ± 1.8	87 ± 2.3
Millet	Zongokolo	89 ± 3.9	85 ± 2.5
Maize†	Yellow dent	82	82
Sorghum	P721-N	82 ± 0.2	56 ± 0.2
Sorghum	P721-Q	88 ± 0.9	73 ± 0.9

*Mean of four determinations.
†Ref. 7.

Table 3. Percent total nitrogen in Landry–Moureaux fractions of raw and cooked millet and sorghum

Fraction		Extraction solvent	% total nitrogen			
			Millet		Sorghum	
			Kordofani	Zongokolo	P-721N	P-721Q
I	Raw	0.5 M NaCl	28	29	17	24
	Cooked		10	11	14	10
II	Raw	70% 2-propanol	40	38	15	6
	Cooked		0	0	0	0
III	Raw	70% 2-propanol/2-ME*	5	0	21	11
	Cooked		9	4	9	18
IV	Raw	pH 10 buffer/2-ME	5	9	7	7
	Cooked		5	7	5	5
V	Raw	pH 10 buffer/2-ME/NaDodSO$_4$	11	16	31	45
	Cooked		56	62	50	50
VI	Raw	Nonextractable	5	5	4	3
	Cooked		20	16	18	13
% recovery		raw	94	97	95	96
		cooked	100	100	96	96

*ME, 2-mercaptoethanol.

Table 4. Percent total nitrogen in Landry–Moureaux fractions of pepsin-indigestible residue

Fraction		% total nitrogen			
		Millet		Sorghum	
		Kordofani	Zongokolo	P-721N	P-721Q
I	Raw	2	0	0	0
	Cooked	2	2	1	1
II	Raw	1	7	4	2
	Cooked	0	0	0	0
III	Raw	0	0	9	5
	Cooked	8	4	8	4
IV	Raw	0	0	3	3
	Cooked	2	3	4	3
V	Raw	3	4	9	9
	Cooked	13	17	30	28

(14). We hypothesized that if higher levels of fraction III proteins are responsible for reduced digestibility after cooking, then pearl millet should resemble corn and be less affected than sorghum by the cooking procedure. Table 2 shows that this hypothesis is correct. The trend in *in vitro* protein digestibility in millet resembles that of corn rather than sorghum in that pepsin digestibility values on individual millet samples decreased upon cooking only slightly if at all. Digestibility values for the two pearl millet varieties studied (Kordofani and Zongokolo) were >25% higher than for normal sorghum. High-lysine sorghum (P-721 opaque) was significantly superior in *in vitro* pepsin digestibility compared with normal sorghum when cooked. Though not previously reported, the higher protein digestibility of cooked high-lysine P-721 opaque sorghum gruel over normal sorghums presented in Table 2 has been consistently observed in this laboratory. MacLean *et al.* (1) also have shown better nitrogen absorption and retention of P-721 opaque in nitrogen-balance studies on preschool children fed with P-721 opaque high-lysine and normal sorghum varieties.

Table 3 shows the effect of cooking on solubility of the proteins in pearl millet, normal sorghum, and high-lysine sorghum lines. Upon cooking, the proportions of fractions I and II were drastically reduced in both millets and sorghum. Whereas fraction III was also decreased by about 50% in normal sorghum, there actually was an increase of fraction III in millets and high-lysine sorghum. Changes in fraction IV were small in both sorghums and millets. Cooking also resulted in a large increase in fraction V of pearl millet (>50%), and normal sorghum (19%) but only 5% in P-721 opaque, high-lysine sorghum. Nonextractable proteins (fraction VI) increased by ≈4-fold in millets as well as sorghum. The pronounced shifts in fraction II and fraction V of pearl millet varieties as a result of cooking resemble those of maize reported by Hamaker *et al.* (7). The protein profiles of the pepsin-indigestible residues of the uncooked and cooked millet and sorghum are shown in Table 4. The overall profiles are similar except in fraction V, where the level of protein in millets is only half that of both normal and high-lysine sorghums.

Table 5 shows the amino acid compositions of whole-grain samples of two varieties of pearl millets, normal sorghum, high-lysine sorghum, normal maize, opaque-2 maize, barley, wheat, and rice. These major cereal foods of the world and their mutant types have basically similar amino acid profiles except for lysine, tyrosine, leucine, isoleucine, and tryptophan. The level of lysine, an essential amino acid, in pearl millet is higher than that in normal sorghum and normal maize and is comparable to that in high-lysine sorghum and opaque-2 maize. The level of tryptophan in pearl millet is probably close to that in high-lysine sorghum based on a calculated value from its fractions. The tyrosine level in pearl millet is lower than that in sorghum, maize, and rice but is comparable to that in barley and wheat. The isoleucine/leucine ratio in pearl millet is lower than that in sorghum and maize and compares favorably to the ratio in small grains (wheat, barley, and rice). This favorable amino acid balance with a high level of essential amino acids, coupled with the superior

Table 5. Amino acid composition in whole-grain samples of pearl millet, sorghum, maize, barley, wheat, and rice varieties

Analysis	Millet		Sorghum		Maize		Barley (Jefferson)	Wheat (winter, soft)	Rice (market)
	Kordofani	Zongokolo	P-721N	P-721Q*	Flint	Opaque-2†			
Protein, %	11.7	9.5	10.5	10.6	9.06	10.6	10.0	10.0	6.7
Amino acids, g/100 g of protein									
Aspartic acid	8.7	8.9	6.7	7.5	6.7	8.4	6.3	5.5	9.8
Threonine	4.2	4.4	3.3	3.3	3.8	3.9	3.9	3.2	3.9
Serine	5.3	5.2	4.6	4.2	5.3	4.9	4.9	5.1	5.4
Glutamic acid	22.1	20.8	21.9	20.1	22.5	19.1	28.1	32.9	19.1
Glycine	3.2	3.7	3.2	3.5	3.6	4.0	4.7	4.6	5.0
Alanine	8.8	8.5	9.3	8.4	8.6	6.9	4.4	4.0	6.1
Cystine	1.2	0.9	1.4	1.5	1.9	2.3	1.4	2.1	1.4
Valine	6.0	6.0	5.1	5.1	5.2	4.9	5.4	4.8	6.5
Methionine	2.3	2.9	1.8	1.6	2.3	2.0	2.1	1.9	3.2
Isoleucine	4.4	4.3	3.8	3.9	3.7	3.9	3.5	3.5	4.1
Leucine	11.5	10.9	14.0	12.2	15.2	11.6	7.7	7.3	8.9
Tryptophan‡	—	—	0.8	1.0	0.6	1.0	—	—	—
Tyrosine	2.4	2.6	3.5	4.2	3.9	4.7	2.8	2.8	3.7
Phenylalanine	5.6	5.3	5.4	4.9	5.5	4.9	5.7	4.8	5.5
Histidine	2.4	2.4	2.2	2.3	3.1	3.3	2.4	2.5	2.7
Lysine	2.8	3.2	2.1	2.9	2.4	3.3	3.9	3.5	4.0
Ammonia	1.3	1.2	1.2	3.2	1.2	3.4	1.3	1.5	1.1
Arginine	3.9	4.2	3.5	4.5	3.7	5.1	4.6	4.9	7.9
Proline	6.8	6.2	8.6	7.6	10.8	9.3	12.7	10.8	4.8
Total	102.3	101.3	101.2	100.7	108.8	106.6	105.3	104.8	102.7

*Ref. 14.
†Ref. 15
‡Ref. 16.

in vitro pepsin digestibility values, suggests that pearl millet is a nutritious and well-digested source of calories and protein for humans.

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