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Seed protein of millets: amino acid composition, proteinase inhibitors and in-vitro protein digestibility

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Six varieties of common millet (*Panicum miliaceum*), three varieties of finger millet (*Eleusine coracana*) and four varieties of foxtail millet (*Setaria italica*) were analyzed to determine the nitrogen constituents, amino acid composition, proteinase inhibitors and in-vitro protein digestibility (IVPD). The non-protein N accounted for 17·3, 12·5 and 17·0% of the total N found in common millet, finger millet and foxtail millet, respectively. Millet proteins were deficient in lysine, but contained adequate levels of the other essential amino acids. The proteins in finger millets were better balanced compared to those in common millet and foxtail millet. Little varietal differences were observed within millet types in terms of amino acid concentrations. The anti-tryptic activities of millets were high compared to their anti-chymotryptic activities. Foxtail millet had no detectable anti-chymotryptic activity. The IVPD values of raw, uncooked millets were low, but were improved by cooking.

INTRODUCTION

Millets form a major part of the staple food of the population in the semi-arid regions of the tropics. Though they contribute significantly to the protein nutrition in these areas, aspects related to the quality of their seed protein have not been fully evaluated. Compared to other cereals, published data on the protein of millets are scanty. For this reason, nitrogen constituents, amino acids composition, proteinase inhibitors and in-vitro protein digestibility of three utricle type millets, namely common millet (*Panicum miliaceum*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), were investigated in the present study.

MATERIALS AND METHODS

Materials

The 13 samples studied represented six varieties of common millet, three varieties of finger millet and four varieties of foxtail millet. The sources of samples and method of sample preparation have been reported earlier (Ravindran, 1991).

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Methods

Total nitrogen was determined by the micro-Kjeldahl procedure (AOAC, 1975). Non-protein nitrogen (NPN) was extracted from the samples using 10% trichloroacetic acid (Singh & Jambunathan, 1981) and the nitrogen content in the supernatant was determined by the micro-Kjeldahl procedure.

The nitrogen solubility was determined by the method of Saunders *et al.* (1974). In this method, 0·5 g of each sample was suspended on 25 ml of distilled water and the pH was adjusted to 2, 4, 6, 8 and 10. The samples were shaken in a water bath at 25°C for 1 h. The sample volume was made up to 25 ml and then centrifuged at 6000 rpm for 15 min. Nitrogen was determined in 5 ml of the supernatant using the micro-Kjeldahl method.

Amino acid composition was determined on 50 mg samples that were hydrolyzed with 6 N hydrochloric acid at 110°C for 24 h. Cystine and methionine were determined as cysteic acid and methionine sulfone, respectively, following oxidation with performic acid (Moore, 1963). The hydrolyzates were analyzed with an automatic amino acid analyzer (LKB model 4151, Alpha Plus), using the hydrolyzate analysis program

described in the apparatus manual. Threonine and serine values were corrected 4 and 10%, respectively, for destruction during acid hydrolysis.

Samples defatted with hexane for 16 h were used for the protease inhibitor analysis. Tryptic and chymotryptic activities were determined by the method of Kakade *et al.* (1969) and Kakade *et al.* (1970), respectively. The trypsin and chymotrypsin inhibitors were extracted with phosphate buffer (0·1 M, pH 7·6) and incubated with enzyme (40 μg trypsin or 24 μg chymotrypsin) and casein at 37°C. Incubation times for trypsin and chymotrypsin were 20 and 10 min, respectively. The absorbance was read in a Spectronic 710 spectrometer (Bausch & Lomb, New York, USA). One trypsin unit (TU) or chymotrypsin unit (CU) is defined as in increase of 0·01 absorbance unit at 280 or 275 nm, respectively. The trypsin inhibitory units (TIU) or chymotrypsin inhibitory units (CIU) were expressed as numbers of TU or CU inhibited per gram dry weight.

In-vitro protein digestibilities (IVPD) of flour samples were estimated by the method of Hsu *et al.* (1977), as modified by Salgo *et al.* (1985). IVPD was also determined on flour samples prepared from heated millet grains.

RESULTS AND DISCUSSION

Data on total N and NPN of millets are summarized in Table 1. The differences in total N between and within millet types are well documented and have been discussed elsewhere (Ravindran, 1991).

Table 1. Total nitrogen and non-protein nitrogen contents of common, finger and foxtail millets[a]

Varieties	Total N (%)	NPN (%)	NPN as % of total N
Common millet			
BR 7	2·4	0·4	18·0
Heen mineri	2·0	0·4	18·7
IPM 1006	2·3	0·4	16·5
MS 2420	2·1	0·4	17·7
MS 4872	2·4	0·4	16·3
Raum 1	2·6	0·4	16·7
Average ± SE	2·3 ± 0·1	0·4 ± 0·0	17·3 ± 0·4
Finger millet			
CO 10	1·5	0·2	12·3
KM 1	1·7	0·2	12·0
MI 302	1·5	0·2	13·2
Average ± SE	1·6 ± 0·06	0·2 ± 0·0	12·5 ± 0·4
Foxtail millet			
KHS 1	2·5	0·5	19·1
SIC 1	2·4	0·4	17·1
SIC 7	2·7	0·4	15·9
SIC 15	2·6	0·4	16·1
Average ± SE	2·5 ± 0·1	0·4 ± 0·03	17·0 ± 0·7

[a] Each value in this table and the following tables is a mean of three determinations.

The NPN contents of finger millets were lower (0·2%) than common millet (0·4%) or foxtail millet (0·4%), and they also had the lowest total N. Comparable data on the NPN contents of millets are scanty, but the present values for finger millets are higher than those reported by Pore and Magar (1979). The NPN accounted for 17·3, 12·5 and 17·0% of the total N found in common millet, finger millet and foxtail millet, respectively. The relatively large NPN components indicate that protein contents of millets may be overestimated by 1–3%, leading to erroneous projection of protein intake in millet-based diets.

Little or no differences were observed in the NPN content among varieties within millet types. This is in contrast to the marked varietal differences noted in terms of total N. No significant correlation was found between the total N and NPN content of the millets.

The nitrogen solubility data of millet flours at various pH values are presented in Table 2. Nitrogen solubilities of all millet flours were low. Nitrogen solubility values were highest at pH 10 and lowest at pH 4 in all cases. The anti-proteinase activities in millet flours may be, at least in part, responsible for the low solubility values. The values might have been higher, if the millets had been heat-treated before milling.

Data on amino acid composition in the present study (Table 3) are in fairly good agreement with previously published reports on foxtail millet (Taira, 1968), finger millet (Pore & Magar, 1979) and common millet (Lorenz & Dilsaver, 1980). Overall, all millets had somewhat similar amino acid profiles. The non-essential amino acids such as glutamic acid, alanine, proline, serine and aspartic acid were the major constituents. Of the essential amino acids, leucine, phenylalanine and valine were present in significant amounts.

In general, the varietal differences in terms of amino acid concentration were of small magnitude. Taira (1968), analyzing 10 varieties of foxtail millet, similarly reported no varietal effects on the amino acid composition.

The essential amino acid patterns of millets as compared to rice, maize and FAO/WHO (1973) reference protein are shown in Table 4. The comparison indiates that, in common with other cereals, lysine is the first limiting amino acid in millets. Tryptophan was not determined in the present study. However, tryptophan is the second limiting amino acid in most cereals and this may be true for millets as well. But several reported indicate millets to contain adequate levels of tryptophan (Taira, 1968; FAO, 1972). Threonine is not a limiting amino acid in millets, in contrast to rice, sorghum and wheat (FAO, 1972). The essential amino acid contents of millets were comparable to those of rice and maize (Table 4), but the lysine contents were lower and the methionine contents were higher. Among the millets, finger millet protein was relatively better balanced; it contained more lysine, threonine and valine.

Table 2. Nitrogen solubilities (%) of common, finger and foxtail millets

Varieties	pH				
	2	4	6	8	10
Common millet					
BR 7	13·5	10·8	12·6	22·8	23·2
Heen mineri	11·2	10·1	12·3	21·0	21·0
IPM 1006	12·4	10·5	12·6	19·8	20·3
MS 2420	11·8	10·8	11·7	19·7	21·3
MS 4872	12·9	11·0	12·1	19·9	20·8
Raum 1	13·8	10·9	12·6	22·5	24·0
Average ± SE	12·6 ± 0·4	10·7 ± 0·1	12·3 ± 0·1	21·0 ± 0·6	21·8 ± 0·6
Finger millet					
CO 10	10·7	7·3	10·1	18·3	20·0
KM 1	10·8	7·8	10·3	19·0	19·0
MI 302	10·7	7·4	10·7	18·4	19·3
Average ± SE	10·7 ± 0·0	7·5 ± 0·2	10·4 ± 0·2	18·9 ± 0·2	19·4 ± 0·3
Foxtail millet					
KHS 1	13·5	11·3	12·8	22·2	24·1
SIC 1	13·5	11·2	12·7	22·5	25·1
SIC 7	13·7	11·7	12·1	22·5	25·8
SIC 15	13·8	11·8	12·9	23·1	25·2
Average ± SE	13·6 ± 0·1	11·5 ± 0·1	12·6 ± 0·2	22·6 ± 0·2	25·0 ± 0·3

Table 3. Protein content[a] and amino acid composition[b] of some varieties of common, finger and foxtail millets

Amino acid	Common millet		Finger millet		Foxtail millet	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Aspartic acid	6·6–6·9	6·7 ± 0·05	6·7–7·5	7·2 ± 0·24	7·7–8·1	7·9 ± 0·12
Threonine	3·7–4·5	4·1 ± 0·14	5·1–5·4	5·2 ± 0·10	4·5–4·6	4·5 ± 0·03
Serine	7·3–7·9	7·6 ± 0·11	6·5–6·8	6·6 ± 0·09	6·0–6·2	6·1 ± 0·05
Glumatic acid	24·9–25·4	25·2 ± 0·09	22·5–26·1	24·2 ± 1·04	23·5–24·3	23·9 ± 0·17
Proline	7·6–7·9	7·8 ± 0·05	7·4–7·8	7·6 ± 0·12	8·0–8·4	8·2 ± 0·09
Glycine	2·8–3·1	2·9 ± 0·05	4·1–4·8	4·5 ± 0·21	3·0–3·4	3·2 ± 0·09
Alanine	10·9–12·4	11·7 ± 0·26	7·1–7·3	7·2 ± 0·06	10·5–10·9	10·7 ± 0·08
Cystine	0·9–1·1	1·0 ± 0·02	1·3–1·6	1·4 ± 0·09	1·0–1·1	1·1 ± 0·03
Valine	6·0–7·3	6·4 ± 0·26	8·0–8·3	8·2 ± 0·09	6·1–6·5	6·3 ± 0·09
Methionine	3·4–4·3	4·1 ± 0·29	4·2–5·2	4·5 ± 0·32	3·8–4·2	4·0 ± 0·10
Isoleucine	4·7–5·4	4·9 ± 0·12	5·1–5·3	5·2 ± 0·06	5·0–5·2	5·1 ± 0·03
Leucine	13·0–14·7	14·0 ± 0·31	11·3–12·0	11·7 ± 0·21	15·4–16·3	16·0 ± 0·19
Tyrosine	4·3–4·7	4·5 ± 0·57	4·0–4·3	4·2 ± 0·09	3·7–3·9	3·8 ± 0·04
Phenylalanine	6·2–6·5	6·3 ± 0·05	6·0–6·2	6·1 ± 0·09	6·2–6·3	6·2 ± 0·03
Histidine	2·3–2·5	2·4 ± 0·03	2·8–2·9	2·8 ± 0·02	2·3–2·4	2·3 ± 0·03
Lysine	1·6–1·9	1·7 ± 0·06	2·8–3·5	3·1 ± 0·20	1·9–2·2	1·9 ± 0·08
Arginine	4·0–4·3	4·1 ± 0·04	4·5–5·5	4·9 ± 0·29	3·7–4·2	3·9 ± 0·11
Protein	12·3–16·3	14·4 ± 1·58	9·2–10·6	9·8 ± 0·42	14·8–16·6	15·9 ± 0·42

[a] % (N × 6·25) on dry basis.
[b] g/100 g protein.
[c] Tryptophan not determined.

The proteinase inhibitory activities of millets are summarised in Table 5. The anti-tryptic activities of all three millets were high compared to their anti-chymotryptic activities. The anti-tryptic activities of common millets were higher than those of finger millet and foxtail millet. Marked differences in anti-chymotryptic activities were also observed among millets. The anti-chymotryptic activity of common millet (62 CIU) was lower than that of finger millet (182 CIU). Foxtail millet had no detectable anti-chymotryptic activity under the assay conditions used. The CIU values obtained for finger millets are comparable, whilst the TIU values are higher than those reported by Veerabhadrappa *et al.* (1978).

The existence of considerable varietal differences in the proteinase inhibitor activity of millets has been previously demonstrated (Chandrasekher *et al.*, 1982). The

Table 4. Essential amino acid patterns (g/16 g N) of millets compared to those of rice, maize and FAO/WHO reference protein

Amino acid	Common millet[a]	Finger millet[a]	Foxtail millet[a]	Rice[b]	Maize[b]	FAO/WHO (1973) reference protein
Isoleucine	4·9	5·2	5·1	4·6	5·1	4·00
Leucine	14·0	11·7	16·0	8·6	12·4	7·00
Lysine	1·7	3·1	1·9	3·9	3·4	5·50
Methionine	4·1	4·5	4·0	2·3	2·2	—
Cystine	1·0	1·4	1·1	1·2	0·9	—
Total sulfur-containing amino acids	5·1	5·9	5·1	3·5	3·1	3·50
Phenylalanine	6·3	6·1	6·2	5·4	5·2	—
Tyrosine	4·5	4·2	3·8	2·8	2·4	—
Total aromatic amino acids	10·8	10·3	10·0	8·2	7·6	6·00
Tryptophan	ND[c]	ND	ND	1·1	0·7	1·00
Threonine	4·1	5·2	4·5	3·8	4·2	4·00
Valine	6·4	8·2	6·3	5·6	5·6	5·00
Arginine	4·1	4·9	3·9	7·9	5·3	—
Histidine	2·4	2·8	2·3	2·6	3·7	—

[a] Present study.
[b] FAO (1972).
[c] Not determined.

results of the present study also lend support to this view.

The in-vitro digestibility of protein in raw, uncooked millets was low (Table 6). The IVPD values of common millet, finger millet and foxtail millets were 71·3, 72·3 and 77·1, respectively. Cooking improved IVPD in all three millets, indicating that the low protein digestibility in uncooked materials is largely due to the presence of heat-labile anti-proteinase factors. The IVPD values of cooked millets are comparable to those reported for other cereals (Khoi *et al.*, 1987).

The IVPD values of cooked finger millet (average, 85·5%) were lower than those for cooked samples of common millet (average, 88·6%) and foxtail millet (average, 91·6%). These relatively low values of finger millet may be reflective of the presence of tannins. The finger millet varieties evaluated in the present study were dark-coloured grains and used with husk, whereas

Table 5. Trypsin and chymotrypsin inhibitory activity of common, finger and foxtail millets

Varieties	TIU[a]	CIU[b]
Common millet		
BR 7	985	53
Heen mineri	926	72
IPM 1006	733	68
MS 2420	385	42
MS 4872	965	84
Raum 1	398	49
Average ± SE	732 ± 113·7	62 ± 6·6
Finger millet		
CO 10	750	178
KM 1	514	183
MI 302	503	186
Average ± SE	589 ± 80·7	182 ± 2·3
Foxtail millet		
KHS 1	482	—
SIC 1	483	—
SIC 7	250	—
SIC 15	830	—
Average ± SE	511 ± 119·5	—

[a] Trypsin inhibitory units per gram flour (dry weight).
[b] Chymotrypsin inhibitory units per gram flour (dry weight).

Table 6. Per cent in-vitro protein digestibility (IVPD) of common, finger and foxtail millets

Varieties	IVPD (%)	
	Raw	Cooked
Common millet		
BR 7	68·4	88·1
Heen mineri	70·1	89·4
IPM 1006	72·2	89·0
MS 2420	73·2	89·8
MS 4872	71·1	86·4
Raum 1	72·9	88·9
Average ± SE	71·3 ± 0·7	88·6 ± 0·5
Finger millet		
CO 10	67·4	86·3
KM 1	74·7	84·7
MI 302	74·7	85·4
Average ± SE	72·3 ± 2·4	85·5 ± 0·5
Foxtail millet		
KHS 1	76·7	91·2
SIC 1	77·0	91·0
SIC 7	79·3	93·8
SIC 15	75·5	90·4
Average ± SE	77·1 ± 0·8	91·6 ± 0·7

all others were light-coloured and dehusked. In general, dark-coloured grains are known to contain high levels of tannins (Hulse *et al.*, 1980).

The overall results indicate that millets are fair sources of essential amino acids, except lysine. However, finger millet is a better source of balanced protein than common millet and foxtail millet. Despite the presence of tannins and protease inhibitors, the protein digestibility of cooked millets compares closely with other cereal grains. In areas where millets form the major part of the diet, they can contribute significantly to protein nutrition.

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