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## Extraction, Characterization and Nutritional Properties of Two Varieties of Defatted Foxtail Millet Flour (*Setaria italica* L.) grown in China

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**Abstract:** In this study, we examined the various protein fractions and protein concentrates of two selected varieties (white and yellow) of foxtail millet grown in China. Characterized by amino acid analysis, differential scanning calorimetry (DSC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein content for the white increased after defatting though not significantly different ( $p < 0.05$ ) from 11.50 to 11.59 and the yellow decreased from 11.41 to 11.39. Fat content shows a significant decreased ( $p < 0.05$ ) after defatting from 2.38 to 0.41 (white) and 2.90 to 0.66 (yellow). Prolamin yellow and glutelin white were the major fractions (38.8 and 47.2%, respectively), followed by albumin yellow and white as 2.6 and 1.5%, respectively and globulin yellow and white as 2.5 and 1.4%, respectively and the difference was significant ( $p < 0.05$ ) among the various protein fractions. Results showed a significant amount of amino acids with essential amino acids above the recommended amount by FAO/WHO for humans. Albumin white possessed the highest DSC result ( $T_p = 79.583^\circ\text{C}$ ,  $\Delta H = 5.115 \text{ J g}^{-1}$ ), glutelin white the lowest ( $T_p = 66.682^\circ\text{C}$ ,  $\Delta H = 0.313 \text{ J g}^{-1}$ ). Fractions and concentrates had molecular sizes below 14.0 and above 97.0 kDa. Protein fractions and concentrates are potential as functional food ingredient.

**Key words:** Foxtail millet, protein concentrate, amino acids, thermal properties, SDS pattern

### INTRODUCTION

Millet is a general term for a wide range of cereals. The name millet is used to describe seeds from several taxonomically divergent species of grass. They are grown mostly in marginal areas and under agricultural conditions in which major cereals fail to give substantial yields (FAO, 2008). Millets are thought to have been among the first cultivated crops, being one of the staple foods in central, Eastern Asia, Europe (mainly in China, India and Russia) and some parts in Africa during the early ages (Baltensperger, 1996). It is grown quite extensively in India though not of major importance as a food crop. They form the major source of energy and protein for millions of people of low income group in Asia and Africa. Millets are classified with maize, sorghum and Coix (Job's tears) in the grass sub-family Panicoideae. It is an important food in many developing countries because of its ability to grow under adverse weather conditions like limited rainfall.

Foxtail millet (*Setaria italica* L.) is also known as Italian millet. Foxtail millet is one of the world's oldest cultivated crops. Its cultivation is estimated to have started over 4000 years ago (Chang, 1987). It is one of the most important food crops of the Neolithic culture in China. In India, foxtail millet is grown primarily in the hot drought B prone arid and semiarid zones and used mostly for food

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purposes especially by people with low income. Foxtail millet is ranked as second in the world's total production of millets and is an important staple food for millions of people in Europe and Asia (Marathe, 1993). Foxtail Millet is an important cereal and nutritious food in traditional diets, especially for people on the Europe, Asia and Africa continents. The main components of millet include starch, protein, lipid, vitamins and minerals (Usha *et al.*, 1996).

It was further reported that minerals like, magnesium, manganese and phosphorus were significantly in higher amount than the others (Gopalan *et al.*, 1987). Millet in general contains significant quantity of essential amino acids particularly the sulphur contain amino acids (methionine and cysteine). It is also higher in fat content than maize, rice and sorghum Obilana and Manyasa (2002). It contains 12.3% crude protein and 3.3% minerals (Vithal and Machewad, 2006).

The seed protein from millet is responsible for up to 50% of the dry weight of the entire seed and makes a significant contribution to their processing properties and nutritional quality (Shewry and Halford, 2002). Although, millions of hectares of millet are harvested worldwide, providing good source of protein for over 500 million people yet still little is known about its important seed storage proteins (Virk and Mangat, 1997). As millets differ from one another by their appearance, taste, grain quality and morphological behavior, their biochemical composition will also be different in a broad sense. For example, the major storage protein of Foxtail millet is alcohol soluble prolamins (Monterio *et al.*, 1988), whereas in Kodo millet and Barnyard millet the alkali soluble glutelin forms the major storage protein as reported by Sudharshana *et al.* (1988) and Monterio *et al.* (1988).

Although, chemical composition and nutritive values of foxtail millet have been reported Monteiro *et al.* (1982) little information is available on the extraction, characterization and nutritional properties of foxtail millet grown in China. These two varieties of foxtail millet (yellow and white) are used in most traditional homes in Africa, Europe and Asia, but no comprehensive study has been done simultaneously to show their similarities and differences. Therefore, the main objective of this study were to extract, characterize, study the nutritional differences between the two varieties of foxtail and analyzed the amino acid composition, molecular weight distribution of the subunits and their thermal properties.

## MATERIALS AND METHODS

The two varieties of foxtail millet (white and yellow) were purchased from a local market in Wuxi, People's Republic of China. All chemicals used in the experiments were of analytical grade. The foxtail millet (1 kg) was defatted twice with hexane for 8 h at room temperature the defatted Millet flour white (DFMFW) and the defatted Foxtail millet flour yellow (DFMFY) were air-dried for 24 h under a vacuum drier. The defatted flour was milled using a laboratory-scale hammer miller and the resulting flour was sieved through a 60 mesh screen and stored at 5°C in sealed glass jars until used. This research was conducted in the State Key Laboratory and School of Food Science and Technology Laboratory of Jiangnan University, Wuxi from December 2008 to April 2009.

### Proximate Analysis

The proximate composition of Foxtail Millet Flour White (FMFW), Foxtail Millet Flour Yellow (FMFY), Defatted Foxtail Millet Flour White (DFMFW) and Defatted Foxtail Millet Flour Yellow (DFMFY) was determined according to Ceirwyn (1995). The moisture content was determined by drying in an oven at 105°C until a constant weight was obtained. Ash was determined by weighing the incinerated residue obtained at 525°C after 4 h. Crude fat was extracted by the Soxhlet method with petroleum ether. The crude protein was determined by the micro-Kjeldahl method and a Conversion factor of 6.25 was used to quantify the crude protein content (Tkachuk, 1969). The carbohydrate content was estimated by subtracting the sum of percentage of moisture, crude fat, crude protein and ash contents from 100%.

### **Protein Fractionation**

Proteins were extracted from defatted fox tail millet flour based on their solubility at room temperature (25°C) in water, 5% NaCl, 0.1 M NaOH and 70% ethanol using the procedure of Osborne (1909) with minor modifications. The defatted flour was extracted with 400 mL distilled water with stirring for 4 h and centrifuged at 3000 x g for 30 min to obtain the albumin fraction (supernatant). The residue from this Step was then similarly extracted with 400 mL of 5% NaCl to obtain the globulin fraction. The residue after extraction of globulin was extracted with 0.1 M NaOH (1 h) to obtain the glutelin fraction, while the residue after glutelin extraction was extracted with 70% ethanol to obtain the prolamin fraction. All the extractions were carried out twice. The albumin, globulin, glutelin and prolamin Fractions were then purified by isoelectric precipitation at pH 4.0, 4.0, 4.1 and 2.5, respectively and washed with distilled water. All fractions were freeze-dried using a Christ-Alpha 1-4 Freeze dryer (Biotech International, Germany). The determination of the various protein fractions and concentrates (N H 6.25) was done with a Micro-Kjeldahl method.

### **Preparation of Protein Concentrate**

Defatted millet flour protein concentrate was prepared according to the procedure described by Olayide (2003) with some modifications. The defatted flour was dispersed in distilled water at flour to water ratio of 1:5 (w/v); the pH was adjusted to pH 9.5 with 1 M NaOH and stirred for 3 h at 30°C. The extract was separated by centrifugation at 4000 x g for 30 min. The residues were re-extracted twice more as described above. The extracts were then combined and protein was precipitated by adjusting the pH to 4.2 with 1 M HCl before centrifugation at 4000x g for 20 min. The protein concentrates (precipitate) (pH 4.2) was washed twice with distilled water. It was then resuspended in distilled water and the pH was adjusted to 7.0 with 1 M NaOH prior to freeze-drying. The dry protein (protein concentrates) were stored in airtight glass containers at 30°C for subsequent analyses. The protein content was determined by the Kjeldahl method.

### **Amino Acid Analysis**

For the determination of the amino acids, samples of protein fractions and protein concentrates, 100 mg for all the samples with 5 mL 6 M HCl were added in a 50 mL stopper bottle and sealed. The air was removed by keeping the sample in a vacuum chamber. The sealed samples were placed in an oven at 120°C for 16 h to hydrolyze. After hydrolysis, 5 mL of 2 mM norleucine internal standard was added and the solution was filtered in a 0.2 FL Gelman membrane filter. One milliliter of stock sample was pipetted into a 50 mL borosilicate glass serum bottle and dried in a freeze-drier. One milliliter of sodium diluent buffer (pH 2.2) was added to the freeze-dried residue and transferred to a 1.5 mL micro-centrifuge tube for HPLC analysis. The prepared samples were injected as 2.5-FL volumes and run on a Waters HPLC (Waters Corporation, Milford, Mass., USA) at a flow rate of 0.4 mL min<sup>-1</sup> with a Pickering sodium ion-exchange column of 4 H 150 mm (Pickering Laboratories, Inc.) and sodium eluent (pH 3.15 and 7.40). TRIONE<sup>7</sup> ninhydrin reagent was added with post column instrument (TRIONE<sup>7</sup> ninhydrin derivatization instrument, Pickering Laboratories, Inc.). The light absorbance of amino acids was detected with an UV Visible detector (Pickering Laboratories Inc., Mountain View, Calif., USA) at 570 nm wavelength and the amino acids were quantified by comparing with standard amino acid profiles.

Methionine and cysteine were determined separately by oxidation products according to the per formic acid procedure of Moore (1963) before hydrolysis in 6 M HCl. Tryptophan was determined after alkaline hydrolysis by isocratic ion-exchange chromatography with o-phthalaldehyde derivatization followed by fluorescence detection Ravindran and Bryden (2005). Amino acid composition was reported as g/100 g of protein.

#### Differential Scanning Calorimetry (DSC)

Thermal properties of prolamin, Glutelin, albumin, globulin and protein concentrate were evaluated using Differential Scanning Calorimetry (DSC) (Pyris-I-DSC, Perkin-Elmer Corp., Norwalk, Conn. and USA). Seventy milligram of various samples were dissolved in 1 mL of 0.05 M phosphate buffer (pH 7.0) containing 0.1 M NaCl. The protein solutions (45 FL) were transferred and hermetically sealed in a stainless steel pan. The samples were heated by scanning from 25 to 135°C at a rate of 10°C min<sup>-1</sup> against a reference containing 45 FL buffer without protein in a differential scanning calorimeter (Perkin-Elmer Corp., Norwalk, Conn., USA). The denaturation peak temperature and enthalpy were calculated by a thermal analysis data software program.

#### SDS Page Analysis

The SDS-PAGE was done on 12 separating and 4% stacking gels according to Laemmli (1970) using low molecular weight (14100-97400 Da) markers obtained from Sigma Aldrich (St. Louis, MO). The lyophilized crude extract powder (0.005 g) was dissolved in 1 mL of 20 mM Tris-HCl buffer at pH 7.1. The solution was then centrifuged at 12000 Hg for 2 min to obtain the analytical sample. The purified inhibitor was applied at a concentration of 0.15 mg mL<sup>-1</sup>. Coomassie brilliant blue R-250 was used for staining.

#### Statistical Analysis

Data were analyzed by Analysis of Variance (ANOVA) using SAS statistical software package (v. 8.1, SAS Institute, Cary, NC). The Significant of difference between means were determined by Duncan's Multiple Range Test (DMRT), where (p<0.05) was considered for significant difference. Each value was determined by at least three replicates. Results were given as Mean±SD.

## RESULTS AND DISCUSSION

#### Proximate Chemical Composition

The proximate chemical composition of the various varieties (FMFW, FMFY, DFMTW and DFMTY) was not significantly different (p<0.05) to each other (Table 1). After defatting the protein content for FMFW increased though not significantly different (p<0.05) (11.50 B 11.92). While, the protein content for the FMFY after defatting decreased (11.41- 11.39), the removal of the fat from foxtail millet did not significantly affect the protein content in foxtail millet. Nonetheless our results corroborated the results reported for kodo millet (Sudharshana *et al.*, 1988). Before defatting, the fat content for FMFW and FMFY was 2.38 and 2.90% respectively, but a significant decrease (p<0.05) was observed for DFMTW and DFMTY 0.41 and 0.66%, respectively. The fat content was relatively low when compared to pearl millet (7.6%) and quinoa (6.3%) (Oshodi and Ogungbenle, 1999). It was also observed that the carbohydrates content was significantly higher for all the samples (Table 1). The results from this study were within the range reported for other samples studied (Amado and Arrigoni, 1992). Other researchers found that the carbohydrates were mainly composed of sugars (sucrose and raffinose), fibers, pentosans and starch in wheat germs (Amado and Arrigoni, 1992).

#### Fractionation of Defatted Millet Protein

The results of the extracted protein fractions from defatted millet shows that the protein content fractions (Albumin, Globulin, Glutelin and Prolamin) in DFMTW and DFMTY revealed that prolamin is the major storage protein in the foxtail millet (Table 2). The content varied from 41 and 77.5% of the total protein among the varieties, next to glutelins. Present resulted supported Parvathy and Thayumanavan (1995), who stated that prolamin and glutelin are the predominant protein fractions in millet and sorghum. However, in sorghum, pearl millet and finger millet prolamins and glutelins are

Table 1: Proximate chemical composition of foxtail millet flour and defatted foxtail millet (White and Yellow) (g/100 g, wb)<sup>a</sup>

Samples	Fractions				
	Prolamin	Globulin	Glutelin	Albumin	Protein concentrate
<b>PC (%)</b>					
White	41.4±1.40b	7.0±0.33a	14.0±0.55d	15.7±0.33e	56.1±0.46d
Yellow	77.5±0.36a	8.6±0.32b	60.2±0.41c	20.1±0.27db	63.8±0.34c
<b>TP (%)</b>					
White	25.5±0.51aa	1.4±0.03bc	47.2±0.33c	1.5±0.30bc	-
Yellow	38.8±0.86b	2.3±0.11ac	16.2±0.93d	2.6±0.11a	-

<sup>a</sup>Values are Mean±SD of four determinations. Mean values followed by the same letter in the same column are not significantly different (p<0.05). FMFW: Foxtail millet flour white, FMFY: Foxtail millet flour yellow, DFMFW: Defatted foxtail millet flour white, DFMFY: Defatted foxtail millet flour yellow

Table 2: Distribution of protein fractions

Samples	Protein (N×6.25)	Moisture	Fat	Ash	Carbohydrate
FMFW	11.50±1.08a	10.45±0.14a	2.38±0.03a	0.47±0.03a	75.2
FMFY	11.41±0.15a	10.22±0.13a	2.90±0.35b	0.68±0.04b	74.8
DFMFW	11.92±0.30a	12.23±0.04b	0.41±0.15c	0.44±0.04c	75.9
DFMFY	11.39±0.38a	12.09±0.10c	0.66±0.17c	0.91±0.03c	75.0

<sup>a</sup>Values are Mean±SD of three replicates. For each group, means followed by different the same first letter in the same row are not significantly different (p<0.05). Means with the same second letter are not significantly different. <sup>b</sup>Protein content % = g of proteins in 100 gram of extracted solids. <sup>c</sup>% of total protein = [total proteins (g) of each fraction extracted from 100 g of meal/total proteins (g) of 100 g of defatted millet flour]×100

present in almost equal amounts and constitute two major storage protein fractions (Tatham *et al.*, 1996) and Chandna and Matta (1990). A large amount of carbohydrates and pigments were extracted into the albumin and globulin fractions this might be the reason for their low protein content. The total protein fractions amounted to 75.6 and 59.9% (w/w) of the total protein content for both white and yellow foxtail millet fractions. This result shows a significant difference (p<0.05). The alcohol soluble prolamin accounted for 25.5 and 38.8% of the total proteins among the two varieties (white and yellow), which was higher than that in wheat flour and rice (Padhye and Salunkhe, 1979; Wrigley and Bietz, 1988). The salt-soluble and alkali-soluble fractions for glutelin for both white and yellow were 47.2 and 16.2%, respectively while globulin for white and yellow as 1.4 and 2.3%, respectively. The results are lower than those of Wrigley and Bietz (1988) when they reported on the protein fractions and amino acids in wheat flour. Albumin, globulin, prolamine and glutelin, when compared with the reported values, the relative proportion of prolamin was higher, while globulin was lower (Wrigley and Bietz, 1988). These differences may be due to millet cultivars, extracting procedures and meal preparation methods.

Furthermore, the protein concentrates obtained had a Protein content of 56 and 63% for DFMFW and DFMFY, respectively. This result was significantly difference (p<0.05). Prolamin and glutelin the predominant protein fractions in DFMFW and DFMFY, could also be the main protein fractions of the protein concentrate. Therefore, the preparation of protein concentrate would be an effective method for recovering proteins from defatted millet flour.

### Amino Acid Composition

The amino acid composition of the four fractions and their protein concentrates of the two varieties of millet (DFMFW and DFMFY) were observed to have similar amino acid composition and no significant difference was found among the two varieties (Table 3). The amino acid patterns of the defatted flours were in close agreement with the values reported for pearl millet and sorghum (Ejeta *et al.*, 1987). Amino acid analysis revealed that millet protein is rich in glutamic acid, leucine and alanine. In addition, aspartic acid and lysine were high in the fractions of the two varieties with the exception of prolamin which is deficient in lysine similar to other cereal. Present results were in

Table 3: Comparative amino acid profiles of two varieties defatted foxtail millet flour (DFMF), four protein fractions and their protein concentrates (g/100 g of protein)

Amino acid	DFMF		Albumin		Globulin		Prolamine		Glutelin		Protein concentrate		FAO/WHO /UNU <sup>a</sup>	
	w	y	w	y	w	y	w	y	w	y	w	y	Child	Adult
<b>Essential amino acids</b>														
Isoleucine	4.54	4.28	4.17	4.89	4.12	4.78	4.22	4.76	4.47	4.63	4.50	1.03	2.80	1.30
Leucine	13.02	12.68	7.62	8.57	7.87	9.67	13.59	17.03	10.05	10.11	8.33	7.95	6.60	1.90
Lysine	0.93	1.48	5.71	7.34	4.53	6.31	0.04	0.05	3.89	5.00	6.13	5.99	5.80	1.60
Methionine	2.70	2.85	1.60	2.79	1.65	11.10	6.70	3.87	6.86	3.22	2.33	1.94	2.50 <sup>b</sup>	1.70
Met + Cys	6.06	3.27	2.41	3.61	2.43	12.11	8.13	4.60	8.05	4.14	3.19	2.53		
Phenylalanine	7.66	5.84	4.34	5.17	4.63	5.93	6.29	6.87	4.57	5.25	4.97	4.91	6.30 <sup>c</sup>	1.90
Phe + Tyr	10.58	8.12	10.41	9.03	9.36	11.33	11.96	10.76	10.72	10.03	9.01	8.61		
Threonine	2.73	3.43	4.12	4.78	3.56	4.65	3.59	3.68	4.53	4.59	4.21	3.98	3.40	0.90
Valine	5.53	5.42	6.34	7.93	6.24	7.57	4.39	5.66	5.68	6.86	6.76	6.58	3.50	1.30
Histidine	2.04	1.98	2.83	3.25	2.88	3.49	1.95	1.89	2.41	3.19	3.07	3.28	1.90	1.60
Tryptophan	1.13	1.30	1.61	1.40	1.21	1.55	0.75	1.53	1.02	1.32	1.27	1.11	1.10	0.50
<b>Nonessential amino acids</b>														
Alanine	10.79	8.67	6.25	7.34	5.82	7.24	11.27	11.03	7.77	7.48	6.53	6.25		
Arginine	2.38	2.79	7.39	7.49	8.97	11.32	2.11	1.45	6.53	7.68	9.49	8.88		
Aspartic acid <sup>d</sup>	6.45	7.19	11.21	12.26	10.17	12.08	4.92	6.48	7.69	8.80	9.01	8.38		
Cysteine <sup>e</sup>	3.36	0.42	0.81	0.82	0.78	1.00	1.43	0.74	1.19	0.92	0.86	0.59		
Glutamic acid <sup>f</sup>	23.56	20.51	13.87	15.57	13.80	18.15	26.30	25.28	20.00	17.62	14.77	13.85		
Glycine	2.20	2.71	6.44	6.53	6.73	7.68	19.06	1.38	4.63	5.33	5.41	5.37		
Serine	5.12	4.25	4.17	4.53	4.24	6.05	6.86	5.42	5.55	4.98	4.90	4.53		
Tyrosine	2.92	2.28	6.07	3.86	4.73	5.39	5.67	3.88	6.15	4.78	4.05	3.70		
Proline	5.06	5.17	2.75	3.52	2.50	4.64	5.46	8.06	4.16	3.72	3.26	2.60		

<sup>a</sup>FAO/WHO/UNU energy and protein requirements (1985); Requirements for methionine+cysteine; Requirements for phenylalanine+tyrosine; <sup>d</sup>Aspartic acid+asparagine; <sup>e</sup>Cysteine+cystine; <sup>f</sup>Glutamic acid+glutamine

agreement with values reported by Ejeta *et al.* (1987) and Asiedu *et al.* (1993) but lower than values reported from Sudan by El-Tinay *et al.* (1979). Neucere and Sumrell (1980) indicated that low content of lysine in millet is attributed to the high content of prolamin (fraction) in most varieties. DFMFW and DFMFY, their fractions and protein concentrates have a well-balanced amino acid composition. Moreover, most of the essential amino acids in the protein fractions and protein concentrates were higher than the reference pattern recommended by FAO/WHO/UNU (1985). It was observed from Table 3 that leucine and phenylalanine + tyrosine are in excess amounts in millet protein. Lysine is the first limiting amino acid cereal as it was observed to be low in the present study. Cysteine was lacking in millet protein. This fact indicates that S-S bonds would be absent in millet protein. Compared with DFMFW and DFMFY, isoleucine, valine, tyrosine, methionine, alanine, phenylalanine, leucine and histidine were extracted in great proportion, while a loss of the content of tryptophan, cystine, threonine, arginine and proline was observed in protein concentrates. The loss of tryptophan is probably due to mild acid treatment in the preparation process of protein concentrate, whereas the lower contents of threonine and arginine may have been caused by alkaline processing, which can cause destruction of cystine, threonine, arginine, serine and lysine (Monique *et al.*, 1975). Therefore, effectively controlling the pH value is very important during protein concentrate preparation. The amino acid composition of protein concentrate is generally in accordance with earlier reported by Ge *et al.* (2001) except for the higher contents of methionine, leucine, alanine and aspartic acid and the lower level of arginine, isoleucine, lysine and glutamic acid in our concentrate. Among the four protein fractions, prolamin contained the lowest amount of tryptophan and sulfur-containing amino acids. Also, prolamine had the lowest amount of lysine. The prolamin and glutelin fractions had better-balanced amino acid patterns when compared with the globulin and albumin fractions. Therefore, prolamin, the predominant fraction of DFMFW and DFMFY were the best for healthy food formulation.

Table 4: Distribution of Amino Acid Classified According to similar Chemical properties in the two varieties of Defatted Foxtail Millet Flour White and Yellow (DFMFW and DFMFY), Four Protein Fractions and their Protein concentrates (g/100 g of protein)

Group	DFMF		Albumin		Globulin		Prolamine		Glutelin		Protein concentrate	
	w	y	w	y	w	y	w	y	w	y	w	y
Hydrophobic (nonpolar) <sup>a</sup>	39.15	37.88	39.52	44.77	41.41	59.61	57.10	42.55	43.81	44.41	43.06	37.77
Uncharged polar <sup>b</sup>	14.13	10.37	15.17	13.99	13.30	17.10	17.55	13.72	17.42	15.28	14.02	12.81
Basic <sup>c</sup>	6.22	6.56	19.17	18.69	18.22	23.03	7.82	5.38	16.57	17.46	19.67	18.57
Acidic <sup>d</sup>	30.01	27.70	25.08	27.82	23.97	30.23	31.22	31.76	27.69	26.42	23.78	22.23
Sulfur-containing <sup>e</sup>	6.06	3.27	2.41	3.61	2.43	12.11	8.13	4.60	8.05	4.14	3.19	2.53
Aromatic <sup>f</sup>	11.71	9.42	12.02	10.44	10.56	12.88	12.71	12.28	11.73	11.36	10.29	9.72

<sup>a</sup>Gly, Ala, Val, Leu, Pro, Met, Phe, Trp and Ile; <sup>b</sup>Ser, Thr, Cys and Tyr; <sup>c</sup>Lys, Arg and His; <sup>d</sup>Asp and Glu; <sup>e</sup>Cys and Met; <sup>f</sup>Phe, Tyr and Trp

Table 5: Thermal Properties of Protein Fractions and protein concentrates of two varieties of defatted foxtail millet

Sample	Prolamin		Glutelin		Globulin		Albumin		Protein concentrate	
	W	Y	W	Y	W	Y	W	Y	W	Y
To	76.756	79.045	64.176	75.917	71.779	69.557	77.108	78.716	75.027	70.5210
Tp	77.051	79.398	66.682	77.383	72.100	70.820	79.583	79.206	75.528	71.4760
Te	77.051	79.765	68.674	79.124	72.598	72.054	82.650	79.709	77.191	73.8260
$\Delta H J g^{-1}$	0.028	2.288	5.115	0.313	0.272	0.022	0.291	0.047	0.019	0.0450
Ar mJ	0.359	19.267	24.910	0.485	1.936	0.317	2.517	0.053	0.145	0.0819

<sup>a</sup>To: Start Temperature Peak; Tp: Peak Temperature; Te: End Temperature;  $\Delta H$ : Delta H; Ar mJ: Area

Classifications of amino acids in different groups according to chemical properties are shown in Table 4. Among the four protein fractions and protein concentrates of the two varieties, globulins yellow and prolamin white fractions contained the highest amount of sulfur-containing amino acids, globulin yellow and albumin white have the highest amount of basic amino acids, whereas hydrophobic and aromatic amino acids content were the lowest, while the sulfur-containing amino acids in globulin fraction were the lowest. The prolamine fractions of both varieties contained the highest amount of acidic amino acids, whereas the content of uncharged polar amino acids was found to be very low in prolamin yellow and globulin white. The glutelin yellow and globulin white have the lowest amount of acidic amino acids. The globulin yellow and prolamin white have the highest total aromatic amino acids. There was no significant difference between four protein fractions of both varieties compared with their protein concentrate and the defatted flour.

#### Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry is a rapid, easy and capable technique for supplying both thermodynamic (heat capacity, enthalpy and entropy) and kinetic data (reaction rate and activation energy) on protein denaturation and has been used extensively in various food systems. DSC has been used to study the thermal properties and structural changes of proteins Reaker and Johnson (1995). This method is highly sensitive to conformational changes (Gorinstein *et al.*, 1996). The information on protein thermal properties is useful for food-processing strategies and heat-processing design (Ju *et al.*, 2001). Data from DSC measurements for prolamin, globulin, albumin, glutelin and the protein concentrate for DFMFW and DFMFY are given Table 5. According to the results the samples have varied denaturation temperatures 77.051, 72.100, 79.583, 66.682, 75.528, 79.398, 70.820, 79.206, 77.383 and 71.476°C, respectively. The samples showed progressively lower enthalpies with increasing outlet temperatures. The enthalpy did not differ significantly among the various fractions. Wang *et al.* (1999) reported that the denaturation temperature and enthalpy changes were 83.4°C and 0.96 J g<sup>-1</sup> for rice bran protein concentrate. The enthalpies of the various samples as stated above were 0.028, 2.288, 5.115, 0.313, 0.272, 0.022, 0.291, 0.047, 0.019 and 0.045°C, respectively. In this study, the various fractions were less denatured than similar product reported by Wang *et al.* (1999). Except



glutelin from foxtail millet flour white that is higher than the reported value (Wang *et al.*, 1999). However, the denaturation temperature of the millet globulin fraction was generally consistent with previous findings (84-96, 77 and 88°C) for oats globulin (Harwalkar and Ma, 1987). In addition, our results for the globulin fraction are close to the reported values as compared with other cereal proteins (Harwalkar and Ma, 1987). The result shows that albumin of millet was more heat-sensitive than rice (73.3°C) and oat (87°C) albumins, whereas the enthalpy of millet globulin was lower than those (3.14 J g<sup>-1</sup> and 5.39 cal g<sup>-1</sup>) of rice and oat globulins (Ma and Harwalkar, 1984; Ju *et al.*, 2001). Thus, the structural properties of defatted foxtail millet protein fractions and protein concentrates will be significantly affected if during their preparation the temperature exceed 80°C, this will denature the proteins.

### SDS Page Analysis

SDS-PAGE analysis of the fractions and their concentrates are shown in Fig. 1a and b. There is a streaking in the patterns of the proteins of the fractions and protein concentrates among the two varieties. Albumin and globulin fractions revealed polypeptides of a wide range of molecular weights. A low protein variety, the fractions were found to have a higher concentration of low-molecular weight polypeptides. Several bands were seen at 97.4, 66.2, 43.0, 35.0 and 20.0 kDa among the two varieties.

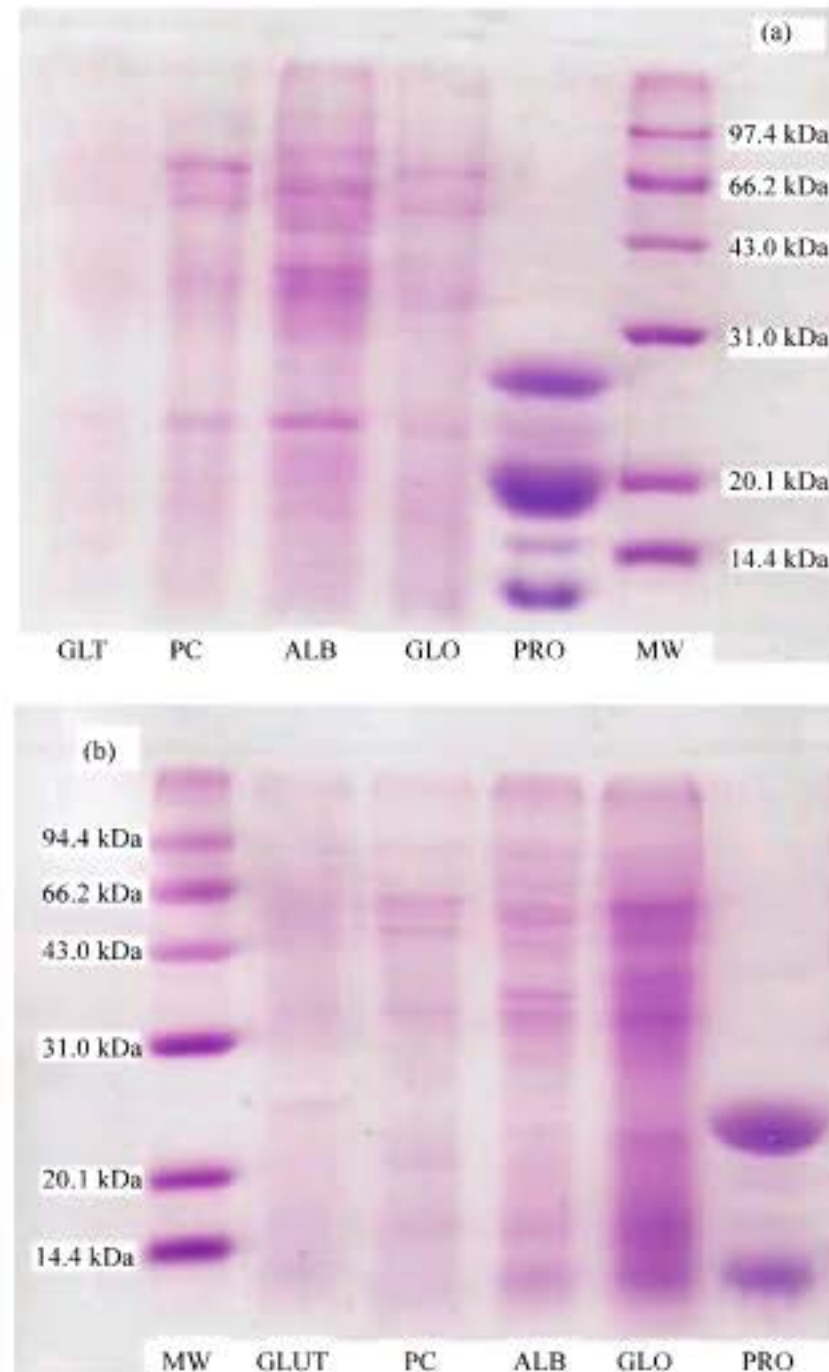


Fig. 1: (a) SDS-PAGE patterns of Yellow foxtail millet and (b) SDS-PAGE patterns of White foxtail millet, MW: molecular weight maker, PRO: prolamin, PC: protein concentrate ALB: albumin GLO: globulin, GLUT: glutelin

This is contrary to kodo millet and barnyard millet which have only four components of albumin and globulin in the range 14.0-32.0 kDa (Monteiro *et al.*, 1988). The SDS-PAGE of prolamins Fig. 1a and b, lane 5 and 6 showed a slight variation among the varieties. Four prominent bands were seen, two below 31.0 kDa, 20 kDa, 14.0 kDa and one prominent band was also seen below 14.0 kDa lane 5 (Fig. 1a). This is similar to the report that prolamins were distributed within the molecular weight range of 27-13 kDa and four to five bands appeared within that range (Parvathy and Thayumanavan, 1995). Nonetheless, however, only two bands appeared within that range in Fig. 1b lane 6. On the contrary, maize contains 10 different polypeptides in the prolamin fraction (Chandna and Matta, 1990). The SDS-PAGE of glutelins Fig. 1a and b revealed many bands throughout the length of the gel and showed no variation among the selected foxtail millet varieties. The SDS-PAGE of the protein concentrates Fig. 1a and b also revealed that many bands run throughout the length of the gel and showed no variation among the yellow and white foxtail millet.

### CONCLUSION

The defatted foxtail millet flour for both varieties contains a moderate amount of protein. Furthermore, the results of the protein characteristics show that millet protein fractions and protein concentrates have potential as a functional food ingredient. The essential amino acid pattern of foxtail millet proteins fractions and their concentrates suggests their possible use as a supplementary protein source to most cereals because this protein is rich in lysine, which is the 1st limiting amino acid for most cereals. They had varied denaturation temperatures and the fractions were found to have a higher concentration of low molecular weight polypeptides. The combined protein fractions and protein concentrates could be used as protein ingredients in food industries.

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### REFERENCES

- Amado, R. and E. Arrigoni, 1992. Nutritive and functional properties of wheat germ. *Int. Food Ingrid.*, 14: 30-34.
- Asiedu, A., E. Lied, R. Nilsen and K. Sandnes, 1993. Effect of processing (Sprouting and/or fermentation) on sorghum and maize: Vitamins and amino acid composition. *Biological utilization of maize protein. Food Chem.*, 48: 201-204.
- Baltensperger, D.D., 1996. Foxtail and Proso Millet. In: *Progress in New Crops*, Janick, J. (Ed.). ASHS Press, Alexandria, VA., pp: 182-190.
- Ceirwyn, S.J., 1995. *Analytical Chemistry of Foods*. Blackie Academic and Professional, London.
- Chandna, M. and K.N. Matta, 1990. Characteristics of pearl millet protein fractions. *J. Biol. Chem.*, 29: 3395-3399.
- Chang, K., 1987. *Archeology of Ancient*. Yale University Press, China.
- Ejeta, G., M.M. Hassen and E.T. Mertz, 1987. *In vitro* digestibility and amino acid composition of pearl millet (*Pennisetum typhoides*) and other cereals. *Proc. Natl. Sci. USA.*, 84: 6016-6019.
- El-Tinay, A.H., A.M. Abdel Gadir and M. El-Hidai, 1979. Sorghum fermented kisra bread. Nutritive value of kisra. *J. Sci. Food Agric.*, 30: 859-863.
- FAO., 2008. *Sorghum and Millets in Human Nutrition*. Food and Agriculture Organization of the United Nations, Rome.

- FAO/WHO/UNU, 1985. Energy and protein requirements. Report of a Joint FAO/WHO/UNU Expert Consultation Technical Report Series No. 724, World Health Organization, Geneva. <http://www.fao.org/docrep/003/AA040E/AA040E00.HTM>.
- Ge, Y., A. Sun, Y. Ni and T. Cai, 2001. Study and development of a defatted wheat germ nutritive noodle. Eur. Food Res. Tech., 212: 344-348.
- Gopalan, C., B.V. Ramasashtri and S.C.N. Balasubramanian, 1987. Nutritive Value of Indian Foods. National Institute of Nutrition, Hyderabad, India, pp: 60.
- Gorinstein, S., M. Zemser, M. Friedman, W.A. Rodrigues, P.S. Martins and N.A. Vello, 1996. Physicochemical characterization of the structural stability of some plant globulins. Food Chem., 56: 131-138.
- Harwalkar, V.R. and C.Y. Ma, 1987. Study of thermal properties of oat globulin by differential scanning calorimetry. J. Food Sci., 52: 394-398.
- Ju, Z.Y., N.S. Hettiarachchy and N. Rath, 2001. Extraction, denaturation and hydrophobic properties of rice flour proteins. J. Food Sci., 66: 229-232.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T<sub>4</sub>. Nature, 227: 680-685.
- Ma, C.Y. and V.R. Harwalkar, 1984. Chemical characterization and functionality J. Agric. Food Chem., 32: 144-149.
- Marathe, J.P., 1993. Structure and Characteristics of the World Millet Economy. In: Advances in Small Millets, Riley, K.W., S.C. Gupta, A. Seetharam and J.N. Mushonga (Eds.). Oxford and IBH, New Delhi, India, pp: 159-178.
- Monique, M., P. Provansal, J.L.A. Cuq and J.C. Cheftel, 1975. Chemical and nutritional modifications of sunflower proteins due to alkaline processing, formation of amino acid cross-links and isomerization of lysine residues. J. Agric. Food Chem., 23: 938-943.
- Monterio, P.V., T.K. Virnepaksha and D.R. Rao, 1982. Proteins of Italian millet: Amino acid composition, solubility fractionation and electrophoresis of protein fractions. J. Sci. Food Agric., 33: 1072-1079.
- Monteiro, P.V., L. Sudharshana and G. Ramachandra, 1988. Japanese barnyard millet (*Echinochloa frumentacea*): Protein content, quality and SDS-PAGE of protein fractions. J. Sci. Food Agric., 43: 17-25.
- Moore, S., 1963. On the determination of cystine and cysteic acid. J. Biol Chem., 238: 235-237.
- Neucere, N.J. and G. Sumrell, 1980. Chemical composition of difference varieties of grain sorghum. J. Agric. Food Chem., 28: 19-21.
- Obilana, A.B. and E. Manyasa, 2002. Millets. In: Pseudo Cereals and Less Common Cereals: Grain Properties and Utilization Potential, Belton, P.S. and J.R.N. Taylor (Eds.). Springer-Verlag, New York, pp: 177-217.
- Olayide, S.L., 2003. Functionality of Africa locus beans (*Parkia biglobosa*) protein isolate: Effect of pH, ionic strength and various protein concentration. Food Chem., 86: 345-355.
- Osborne, T.B., 1909. The Vegetable Proteins. 2nd Edn., Longmans Green, London, pp: 125.
- Oshodi, A.A. and H.N. Ogungbenle, 1999. Chemical composition, nutritionally valuable minerals and functional properties of benniseed, pear millet and quinoa flours. Int. J. Food Sci. Nutr., 50: 325-331.
- Padhye, V.W. and D.K. Salunkhe, 1979. Extraction and characterization of rice proteins. Cereal Chem., 56: 339-389.
- Parvathy, P.K. and B. Thayumanavan, 1995. Homologies between prolamins of different minor millets. Plant Foods Human Nutr., 48: 119-126.
- Ravindran, G. and W.L. Bryden, 2005. Tryptophan determination in proteins and feedstuffs by ion exchange chromatography. Food Chem., 89: 309-314.

- Reaker, M.O. and L.A. Johnson, 1995. Thermal and functional properties of bovine blood plasma and egg white proteins. *J. Food Sci.*, 60: 685-690.
- Shewry, P.R. and N.G. Halford, 2002. Cereal seed storage proteins: Structures, properties and role in grain utilization. *J. Exp. Bot.*, 53: 947-958.
- Sudharshana, L., P.V. Monteiro and G. Ramachandra, 1988. Studies of kodo millet (*Paspalum scrobiculatum*). *J. Sci. Food Agric.*, 42: 315-323.
- Tatham, A.S., R.J. Fido, C.M. Moore, D.D. Quesada, D.D. Kuzmicky, J.N. Keen and P.R. Shewry, 1996. Characterisation of the major prolamins of tef (*Eragrostis tef*) and finger millet (*Eleusine coracana*). *J. Cereal Sci.*, 24: 65-71.
- Tkachuk, R., 1969. Nitrogen-to-protein conversion factors for cereals and oilseed meals. *Cereal Chem.*, 46: 419-424.
- Usha, A., G. SriPriya and T.S. Chandra, 1996. The effect of fermentation on the primary nutrients in foxtail millet (*Setaria italica*). *Food Chem.*, 56: 381-384.
- Virk, D.S. and B.K. Mangat, 1997. Pearl Millet. In: Genetics, Cytogenetics and Breeding of Crop Plants, Bahl, S.P.N. and A.K. Mandal (Eds.). Science Publisher's Inc., Enfield, pp: 241-265.
- Vithal, D.S. and G.M. Machewad, 2006. Processing of foxtail millet for improved nutrient availability. *J. Food Proces. Preser.*, 30: 269-279.
- Wang, M., N.S. Hettiarachchy, M. Qi, W. Burks and T.J. Siebenmorgen, 1999. Preparation and functional properties of rice bran protein isolate. *J. Agric. Food Chem.*, 47: 411-416.
- Wrigley, C.W. and J.A. Bietz, 1988. Proteins and Amino Acids. In: Wheat Chemistry and Technology, Pomeranz, Y. (Ed.). AACC International, St. Paul, MN., pp 159-276.