



American Journal of  
**Food Technology**

ISSN 1557-4571



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## Comparative Study of Chemical Composition and Physicochemical Properties of Two Varieties of Defatted Foxtail Millet Flour Grown in China

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**Abstract:** In this study, we examined the chemical composition and physicochemical properties of two varieties defatted foxtail millet flour grown in China. The seeds were obtained, milled and sieved to produce flour. The flours were tagged DFMTW and DFMTY for defatted foxtail millet flour white and defatted foxtail millet flour yellow, respectively. The protein contents of DFMTW and DFMTY were 11.92 and 11.39, respectively. DFMTY had higher mineral elements, ash and fat content than DFMTW. Essential amino acids were above the recommended amount by Food Agricultural organization/World Health Organization (FAO/WHO) for humans. The foxtail millet flours had molecular sizes below 14.4 kDa and above 97.0 kDa. They had similar solubility curves. Water binding capacity was in the range of 1.36 and 1.26 g g<sup>-1</sup>, while oil absorption capacity ranged between 0.78 and 0.50 g g<sup>-1</sup> for both DFMTW and DFMTY, respectively. A low bulk density (0.27 and 0.23 g mL<sup>-1</sup>) and was also low in total phenolic assay (0.56 and 0.72 mg g<sup>-1</sup>) was observed for both DFMTW and DFMTY, respectively. Foam capacity was 13.36 mL for DFMTW and 12.32 mL DFMTY. Their infrared falls within (1600 and 600 cm<sup>-1</sup>) and both samples possessed O-H and C-H compounds. Defatted foxtail millet flour could be used in food formulation with less fear of retrogradation.

**Key words:** Foxtail millet flour, *in vitro* protein digestibility, protein solubility, molecular size, amino acid

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### INTRODUCTION

The search for lesser known and underutilized crops, many of which are potentially valuable for human and animal consumption. This has intensified to maintain a balance between population growth and agricultural productivity, particularly in the tropical and subtropical areas of the world (Oladele and Aina, 2007). The growing of cereals plays a major role in the agricultural production of the majority of countries. This fact is connected with importance of some cereals in nutrition. The majority of wheat, rice, rye, sorghum and millet

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are used for food. They contribute an important source of protein in the diet in different countries of the world (Shewry and Miflin, 1986). In many developing countries cereal proteins form 70 to 90% of the total protein consumption. Maize, barley and growing parts of other cereals are used in animal feed, especially in developing countries (Shewry and Miflin, 1986; Tatham *et al.*, 1996).

Cereals support half of the daily per capital protein supply in the world. Demand of relatively inexpensive sources of proteins that can be incorporated to value added food products is increasing.

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. Millets are classified with maize, sorghum and Coix (Jobs tears) in the grass sub-family Panicoideae. They are grown mostly in marginal areas and under agricultural conditions in which major cereals fail to give substantial yields. Millets typically contain higher quantities of essential amino acids methionine and cysteine and are higher in fat content than maize, rice and sorghum (Kamara *et al.*, 2009; Obilana and Manyasa, 2002). 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). 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.*, 1982), 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.* (1982).

Foxtail millet (*Setaria italica* L.) is also known as Italian millet. It is one of the worlds oldest cultivated crops. In the northern area of China it has been widely used as a nourishing gruel or soup for pregnant and nursing women and has been applied to food therapy. This millet contains 12.3% crude protein and 3.3% minerals (Vithal and Machewad, 2006). Foxtail Millet is an important cereal and nutritious food in traditional diets, especially for people in Europe, Asia and Africa continents.

The digestibility of the nutrients must be known in order to evaluate fully the significance of nutrient concentration. The chemical composition of cereal grains and their anatomical parts varies with the cultivars, agronomic conditions and soil fertility level, but a generalized composition can be considered for most practical purposes. Functional properties are determined to a large extent by a proteins physicochemical and structural properties. Solubility is an important prerequisite for food proteins functional properties and it is a good index of potential applications of proteins (Kinsella, 1979). Bulk density is an important parameter that determines the packaging requirement of a product (Chandi and Sogi, 2007). Amino acid composition and molecular size are the foundations of protein functionality (Kinsella, 1979). In this study, we intend to comprehensively acquire a positive proof of the relationship reported in our previous studies and to further investigate their *in vitro* protein digestibility, total phenolic assay, mineral analysis, amino acid composition, protein solubility, molecular size, foam, water and oil holding capacity, bulk density and inferred analysis, from two varieties of defatted foxtail millet flour.

## **MATERIALS AND METHODS**

The two varieties of foxtail millet (white and yellow) were purchased from a local market in Wuxi, P.R. 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 May 2009 to August 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 James (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%.

### **Amino Acid Analysis**

For the determination of the amino acids, samples of foxtail millet flour 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 µL 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 µL 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 4×150 mm (Pickering Laboratories, Inc.) and sodium eluent (pH 3.15 and 7.40). TRIONE<sup>®</sup> ninhydrin reagent was added with post column instrument (TRIONE<sup>®</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 performic acid procedure of Moore (1963) before hydrolysis in 6 M HCl. Amino acid composition was reported as g/100 g of protein.

### **Mineral Analysis**

The minerals were analyzed from solutions obtained by first dry ashing the defatted foxtail millet flour at 550°C the ash obtained. The residues of both samples were dissolved in 10 mL of 50% of nitric acid solution and made up to final volume of 25 mL of distilled water. After that the minerals (Zn, Fe, Cu, Mn, Na, K, Mg and Ca) were analyzed separately, using an Atomic Absorption Spectrophotometer of Spectrr AA 220, USA Varian. Phosphorus was analyzed by the phosphovanado molybdate method of AOAC (1995). The data reported represents the average of three determinations.



### Protein Digestibility by Trypsin

*In vitro* protein digestibility was carried out according to the method described by Elkhalil *et al.* (2001) with slight modification. About 20 mg of foxtail millet flour samples in triplicate were digested in 10 mL of trypsin (0.2 mg mL<sup>-1</sup> in 100 mM Tris-HCl buffer, pH 7.6). The suspension was incubated at 37°C for 2 h. Hydrolysis was stopped by addition of 5 mL 50% trichloroacetic acid (TCA). The mixture was allowed to stand for 30 min at 4°C and was then centrifuged at 9500x g for 30 min using a D-3756 Osterode am Harz model 4515 Centrifuge (Sigma, Germany). The resultant precipitate was dissolved in 5 mL of NaOH and protein concentration was measured using the micro-Kjeldahl method. Digestibility was calculated as follows.

$$\text{Protein digestibility (\%)} = \frac{(A-B)}{A} \times 100 \quad (1)$$

Where:

A = Total protein content (mg) in the sample

B = Total protein content (mg) in TCA precipitate

### Total Phenolic Assay

The total phenolic content of foxtail millet flour was determined by using the Folin-Ciocalteu micro method (Singleton and Rossi, 1965). About 20 µL of extracts solution were mixed with 1.16 mL distilled water and 100 µL of Folin-Ciocalteu reagent, followed after 1 min and before 8 min by 200 µL of Na<sub>2</sub>CO<sub>3</sub> solution (20%). Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as standard for calibration curve. The phenolic content was reported as gallic acid equivalents using the following linear equation based on the calibration curve:

$$y = 0.153x - 0.1433, R^2 = 0.99$$

where, y is the absorbance and x is Gallic acid equivalent µL mL<sup>-1</sup>.

### Foam Capacity

The method described by Nanayana and Narasinga Rao (1982) was used for the determination of foam capacity with some modifications. Two milligram of flour sample was added to 50 mL distilled water at 30±2°C was mixed thoroughly using an Ultra-turrax T25 homogenizer at 9500 rpm for 3 min in a 100 mL graduated cylinder and the volume of foam after 30 sec was recorded. The foam capacity is expressed as percentage increase in volume.

### Water/Oil Absorption

Sample (0.5 g) was taken and mixed with 3 mL of distilled Water or refined groundnut oil. The slurry was centrifuged at 750x g for 15 min. The pellet was drained for 30 min and the gain in weight per unit weight was reported as water or oil absorption capacity (g g<sup>-1</sup>), respectively.

### Bulk Density

A known weight of the foxtail millet flour was added to a graduated measuring cylinder. The cylinder was gently tapped and volume occupied by the sample was determined. Bulk density was reported as weight per unit volume (g mL<sup>-1</sup>).

### **Nitrogen Solubility**

Nitrogen solubility was determined according to the procedure of (Bera and Mukherjee, 1989). One hundred milligram of foxtail millet flour for both varieties were dispersed in 10 mL of distilled deionized water. The suspensions were adjusted to pH 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 using either 0.1 M HCl or 0.1 M NaOH. These suspensions were shaken (Lab Line Environ Shaker; Lab Line Instrument, Inc., Melrose Park, Ill., USA) for 30 min at room temperature (approximately 25°C) and centrifuged at 4000x g for 30 min. The nitrogen content of the supernatant was determined by the Kjeldahl method and percent nitrogen solubility was calculated as follows:

$$\text{Nitrogen solubility (\%)} = \frac{\text{PS(mg)}}{\text{PIS(g)}} \times 100 \quad (2)$$

Where:

PS = Amount of protein in supernatant

PIS = Protein in initial sample

### **Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

SDS-PAGE was done on 12% separating and 4% stacking gels according to Laemmli (1970) using low molecular weight (14100-97400) markers obtained from Sigma Aldrich (St. Louis, MO). For lyophilized crude extract powder; the (0.005 g) was dissolved in 1 mL of 20 mM Tris-HCl buffer at pH 7.1. The solution was then centrifuged at 12000x g 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.

### **The Infrared (IR)**

The IR analysis of DFMTW and DFMTY powders were carried out by mixing 0.1 g sample and 0.5 g potassium bromide (KBr) were finely ground. A thin film 1 cm<sup>-1</sup> diameter and uniform thickness was prepared from both varieties of foxtail millet flour on a special apparatus provided for that work. The infrared absorption of the thin film at 1800 to 800 cm<sup>-1</sup> was recorded using a Nicolet 360 Ft-IR spectrometer (USA) to develop the peaks according to the compounds present in both varieties.

## **RESULTS AND DISCUSSION**

### **Proximate Chemical Composition**

The proximate chemical compositions of the various varieties (FMFW, FMFY, DFMTW and DFMTY) were not significantly different ( $p < 0.05$ ) from each other. The defatting process altered the protein content of the flours though not significantly ( $p < 0.05$ ) depending on the variety of foxtail millet. For the white variety an increase was observed from 11.50-11.92 after defatting which was in contrast to a slight decrease from 11.41 to 11.39 for the yellow variety (Table 1). This observation corroborates the results reported for the kodo millet Sudharshana *et al.* (1988) using similar methods. Although, the defatting method used could not significantly enhance the protein content of the millet flours, it however, significantly difference ( $p < 0.05$ ) the fat content from 2.38 to 0.41% and 2.90 to 0.66% in the white and yellow varieties respectively (Table 1). Generally, the fat contents of both defatted foxtail millet varieties were relative lower compared to other millets like pearl millet (7.6%) or quinoa (6.3%) (Oshodi and Ogungbenle, 1999), but with significantly higher carbohydrate contents (Table 1).



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

Sample	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

Values are Mean±SD of four determinations. Mean values followed by the same letters 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. <sup>a</sup>wb: Wet bases

Table 2: Comparative amino acid profiles of two varieties of defatted foxtail millet flour (white and yellow) (g/100 g protein)

Amino acid	DFMFW	DFMFY	FAO/WHO/UNU <sup>a</sup>	
			Child	Adult
<b>Essential amino acid</b>				
Isoleucine (Ile)	4.58	4.59	2.80	1.30
Leucine (Leu)	13.14	13.60	6.60	1.90
Lysine (lys)	0.94	1.59	5.80	1.60
Methionine (met)	2.72	3.06	250 <sup>b</sup>	1.70 <sup>b</sup>
Met + Cys	3.06	3.50		
Phenylalanine (Phe)	7.73	6.27	6.30 <sup>c</sup>	1.90 <sup>c</sup>
Phe + Tyr	10.68	8.71		
Threonine (Thr)	2.76	3.68	3.40	0.90
Valine (Val)	5.58	5.81	3.50	1.30
Histidine (His)	2.06	2.11	1.90	1.60
Tryptophan (trp)	ND <sup>e</sup>	ND	1.10	0.50
<b>Nonessential amino acid</b>				
Alanine (Ala)	10.89	9.30		
Arginine (Arg)	2.40	3.00		
Aspartic acid (Asp) <sup>d</sup>	6.51	7.71		
Cysteine (Cys) <sup>e</sup>	0.34	0.45		
Glutamic acid (Glu) <sup>f</sup>	23.77	22.00		
Glycine (Gly)	2.22	2.91		
Serine (Ser)	5.17	4.56		
Tyrosine (tyr)	2.94	2.44		
Proline (Pro)	5.10	5.54		
TEAA <sup>h</sup>	53.25	52.92		
TAA <sup>i</sup>	112.5	110.83		

<sup>a</sup>FAO/WHO/UNU energy and protein requirements (1995). <sup>b</sup>Requirements for methionine+cysteine. <sup>c</sup>Requirements for phenylalanine+tyrosine. <sup>d</sup>Aspartic acid+asparagines. <sup>e</sup>Cysteine+cystine. <sup>f</sup>Glutamin acid+glutamine. <sup>g</sup>ND: Not determined. <sup>h</sup>TEAA: Total essential amino acid. <sup>i</sup>TAA: Total amino acid. DFMFW: Defatted foxtail millet flour white, DFMFY: Defatted foxtail millet flour yellow

### Amino Acid Analysis

The DFMF and DMFY had very similar amino acid patterns (Table 2). 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). Valine and threonine contents in DFMFW and DFMFY were in accordance with results reported by Kexue *et al.* (2006). But arginine, phenylalanine and leucine contents in DFMFW and DFMFY were higher. Kexue *et al.* (2006) reported a much higher value of lysine content (6.67 g/100 g) in wheat germ flour. Moreover, most of the essential amino acids in both varieties were higher than the reference pattern recommended by (FAO/WHO/UNU, 1985). It was observed from (Table 2) that leucine and phenylalanine+tyrosine are in excess amounts in millet flour. Lysine and cysteine were low in both varieties (Table 2). This fact indicates that S-S bonds would be absent in the structure of foxtail millet flour.

### Mineral Analysis

The mineral compositions of both varieties were significantly different ( $p>0.05$ ) (Table 3). Defatted foxtail millet flour had potassium as the predominant mineral followed by magnesium, calcium and sodium, zinc copper and small trace of phosphorus. The above minerals were found in significantly higher quantity in DFMFY than DFMFW. The minerals varied significantly ( $p>0.5$ ) between the DFMFY and DFMFW. This might be attributed to the type of soil and areas where the seeds are grown or may be some elements were eliminated during dehulling. The values of calcium found in the flours 211.67 and 211.67  $\mu\text{g g}^{-1}$  for DFMFY and DFMFW, respectively, are adequate for bone and teeth development in infants. Phosphorus and calcium occur together in the body to maintain body blood. The presence of other minerals such as iron is highly important because of its requirement for blood formation and copper is an essential and beneficial element in human metabolism.

### In vitro Protein Digestibility (IVPD)

The *in vitro* protein digestibility of the both varieties was significantly different ( $p<0.05$ ) from each other (Table 4). The values for the trypsin digestibility for DFMFW and DFMFY are 51.59 and 49.18, respectively. Our results are lower when compared to Kexue *et al.* (2006). All two samples showed low trypsin digestibility with a significant difference ( $p<0.05$ ). The presence of protease inhibitors, polyphenols and starch in both varieties might be partially related to its low digestibility. DFMFW showed a relatively higher digestibility when compared to DFMFY. Another factor worthy noting is the unfolding of the native protein structure during its hydrolysis.

### Total Phenolic Assay

The total phenolic assay of the DFMFW and DFMFY ranged from 0.56 and 0.72  $\text{mg g}^{-1}$ , respectively (Table 4). But were significantly different ( $p<0.001$ ). The values obtained are

Table 3: Mineral composition of the two varieties of defatted foxtail millet flour grown in china ( $\mu\text{g g}^{-1}$ )

Mineral element ( $\mu\text{g g}^{-1}$ )	DFMFY	DFMFW
Zinc (Zn)	33.400±0.21	28.530±0.45
Iron (Fe)	20.300±0.10	16.430±0.06
Copper (Cu)	7.570±0.21	5.470±0.45
Manganese (Mn)	7.010±0.01	2.830±0.03
Calcium (Ca)	211.670±0.58	183.900±0.85
Potassium (K)	2002.400±0.42	1351.530±2.15
Magnesium (Mg)	983.070±2.60	499.430±1.72
Sodium (Na)	109.170±0.58	65.270±0.38
Phosphorus (P) <sup>a</sup>	0.022±0.001	0.023±0.002

<sup>a</sup>Value are g/100 g of sample. Values are Means±SD of three determinations. Data are statistical differences ( $p>0.05$ ). DFMFW: Defatted foxtail millet flour white, DFMFY: Defatted foxtail millet flour yellow

Table 4: *In vitro* digestibility, foam capacity, water holding capacity, oil holding capacity, bulk density and total phenolic compounds

	DFMFW	DFMFY
<i>In vitro</i> digestibility (%)	51.59±0.90*	49.18±0.66*
Total phenolic assay ( $\text{mg g}^{-1}$ )	0.56±0.02***	0.72±0.02***
Foam capacity (%)	13.36±0.37*	12.32±0.52*
Water holding capacity ( $\text{g g}^{-1}$ )	1.36±0.07**	1.26±0.03**
Oil holding capacity ( $\text{g g}^{-1}$ )	0.78±0.02***	0.50±0.01***
Bulk density ( $\text{g mL}^{-1}$ )	0.27±0.01*	0.23±0.01*

Values are Mean±SD of five determinations. Data are significant differences at \* $p<0.05$ ; \*\* $p<0.01$  and \*\*\* $p<0.001$ . DFMFW: Defatted foxtail millet flour white, DFMFY: Defatted foxtail millet flour yellow



comparable (McDonough and Rooney, 2000; Vithal and Machewad, 2006). Like many other cereal grains, foxtail millet grains have a thick outer layer of pericarp and colored testa, which contains most of the phenols and tannins. During dehulling, as the outer layer of the pericarp was removed, the phenol and tannins were also removed to the extent of 41.49% (Vithal and Machewad, 2006).

#### **Foam Capacity**

The formation of protein based foams involves the diffusion of soluble proteins toward the air water interface and the rapid conformational change and rearrangement at the interface. Stable foam requires the formation of thick, cohesive and viscoelastic film around each gas bubble (Kinsella, 1979). The foaming capacities of the foxtail millet are remarkably low with DFMTW and DFMTY having 13.6 and 12.32 mL, respectively, were significantly different ( $p < 0.5$ ) (Table 4). Nonetheless our results corroborated the results reported for tiger nut flour (Oladele and Aina, 2007). But lower than wheat flour and breadfruit kernel flour reported by Akubor and Badifu (2004). The low foam capacity may be attributed to the low protein content of the flour since foamability is related to the amount of solubilized protein (Nanayana and Narasinga Rao, 1982).

#### **Water/Oil Absorption**

The water absorption capacity of the DFMTW and DFMTY ranged from 1.36 and 1.26  $\text{g g}^{-1}$ , respectively (Table 4). But were significantly different ( $p < 0.01$ ). The values obtained are comparable to Oladele and Aina (2007). Interactions of water and oil with proteins are very important in the food systems because of their effects on the flavor and texture of foods. Intrinsic factors affecting water binding of food protein include amino acids composition, protein conformation and surface hydrophobicity/polarity (Barbut, 1999). In food applications, the water holding capacity or water uptake capacity of a protein is more important than hydration.

Oil absorption capacity DFMTW and DFMTY were significantly different ( $p < 0.001$ ) and were found to have 0.78 and 0.50  $\text{mL g}^{-1}$ , respectively (Table 4). The result shows that foxtail millet flour may be a lower retainer than raw winged bean (Nanayana and Narasinga Rao, 1982). The lower oil absorption capacity of foxtail millet flour might be due to low hydrophobic proteins which show superior binding of lipids (Kinsella, 1976). Further more, high oil absorption is essential in the formulation of food systems like sausages, cake, batters, mayonnaise and salad dressings.

#### **Bulk Density**

DFMTW and DFMTY had similar bulk density of 0.27 and 0.23  $\text{g mL}^{-1}$ , respectively but was significantly different ( $p < 0.05$ ) (Table 4). Present results obtained for DFMTW and DFMTY were lower compared to reported values tigernut flour (Oladele and Aina, 2007). The low bulk density of foxtail millet flour was due to its lower particle density and the large particle size. Bulk density is a measure of heaviness of flour. More over, bulk density is an important parameter that determines the packaging requirement of a product. Further more; Bulk density signifies the behavior of a product in dry mixes. Also, it varies with the fineness of the particles. High bulk density is disadvantageous for the formulation of weaning foods, where low density is required (Onimawo and Egbekun, 2002).

#### **Nitrogen Solubility**

The pH nitrogen solubility profiles of DFMTY and DFMTW had very similar solubility profiles, exhibiting a V-shaped curve in which the DFMTW had higher solubility value at



alkaline pH. In acidic condition, both varieties had solubility (above 10%) at pH 2.0, but DFMFY had lower solubility than DFMFY at pH 4.0 (less than 2%). At pH 6.0 or above, all proteins dissolved between (18 and 40%), with DFMFY having slight higher solubility than DFMFY (Fig. 1). The presence of polyphenols and starch might be partially related to its low solubility. The maximum solubility was in alkaline conditions because most food proteins are slightly acidic with the amount of aspartic acid and glutamic acid residues being greater than that of lysine, arginine and histidine residues (Oshodi and Ogungbenle, 1999).

#### Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

DFMFY and DFMFY revealed polypeptides of a wide range of molecular weights. The foxtail millet flour of both varieties was found to have a higher concentration of low molecular weight polypeptides. The result is consistent with the previous amino acid analysis. The SDS-PAGE pattern of DFMFY and DFMFY were different from each other (Fig. 2). Furthermore, DFMFY and DFMFY possessed similar band patterns, but DFMFY possessed more prominent bands. They shared several groups of bands

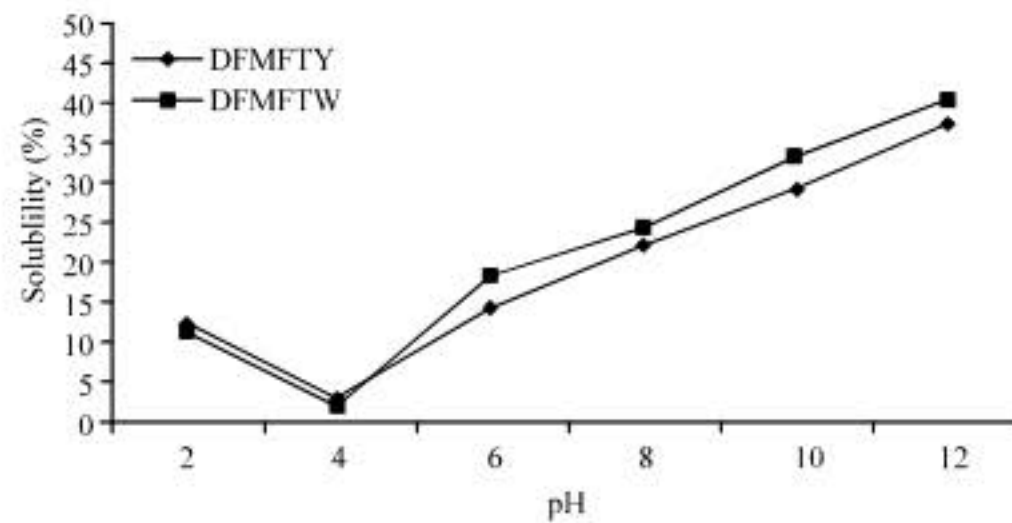


Fig. 1: Effect of pH treatment on nitrogen solubility of defatted foxtail millet flour, DFMFY: Defatted foxtail millet flour white, DFMFY; defatted foxtail millet flour yellow

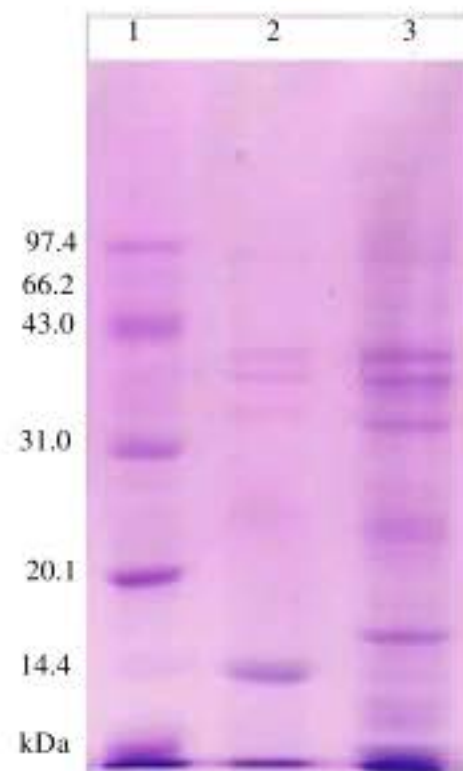


Fig. 2: SDS-PAGE patterns of defatted foxtail millet flour white and yellow Lane 1: Molecular weight maker, Lane 2: Defatted foxtail millet flour white, Lane 3: Defatted foxtail millet flour yellow



between 66.2 to 97 kDa, 31.0 to 43.0 kDa, 20.1 to 31.0 kDa and 14.4 to 20.1 kDa. Some bands were below 14.4 kDa. This is contrary to kodo millet and barnyard millet (Monteiro *et al.*, 1987). The SDS-PAGE of foxtail millet flour also revealed that many bands run throughout the length of the gel and showed slight variation among the white and yellow foxtail millet.

### Infrared Analysis

The IR spectra of DFMTW and DFMTY were shown on (Fig. 3a, b), respectively. IR spectrum determines the presence or absence of particular functional group (Lau, 1999). A comparison of IR spectra of DFMTW and DFMTY determined the structural similarities and differences between the two samples. The DFMTW and DFMTY are different in structures (Fig. 3a, b). Since, each type of covalent has its own characteristic absorption frequencies, no two molecules precisely have the same spectrum (Demirdöven *et al.*, 2004). In general, these differences appear in the within  $1600$  to  $600\text{ cm}^{-1}$ , this region of IR is called the finger

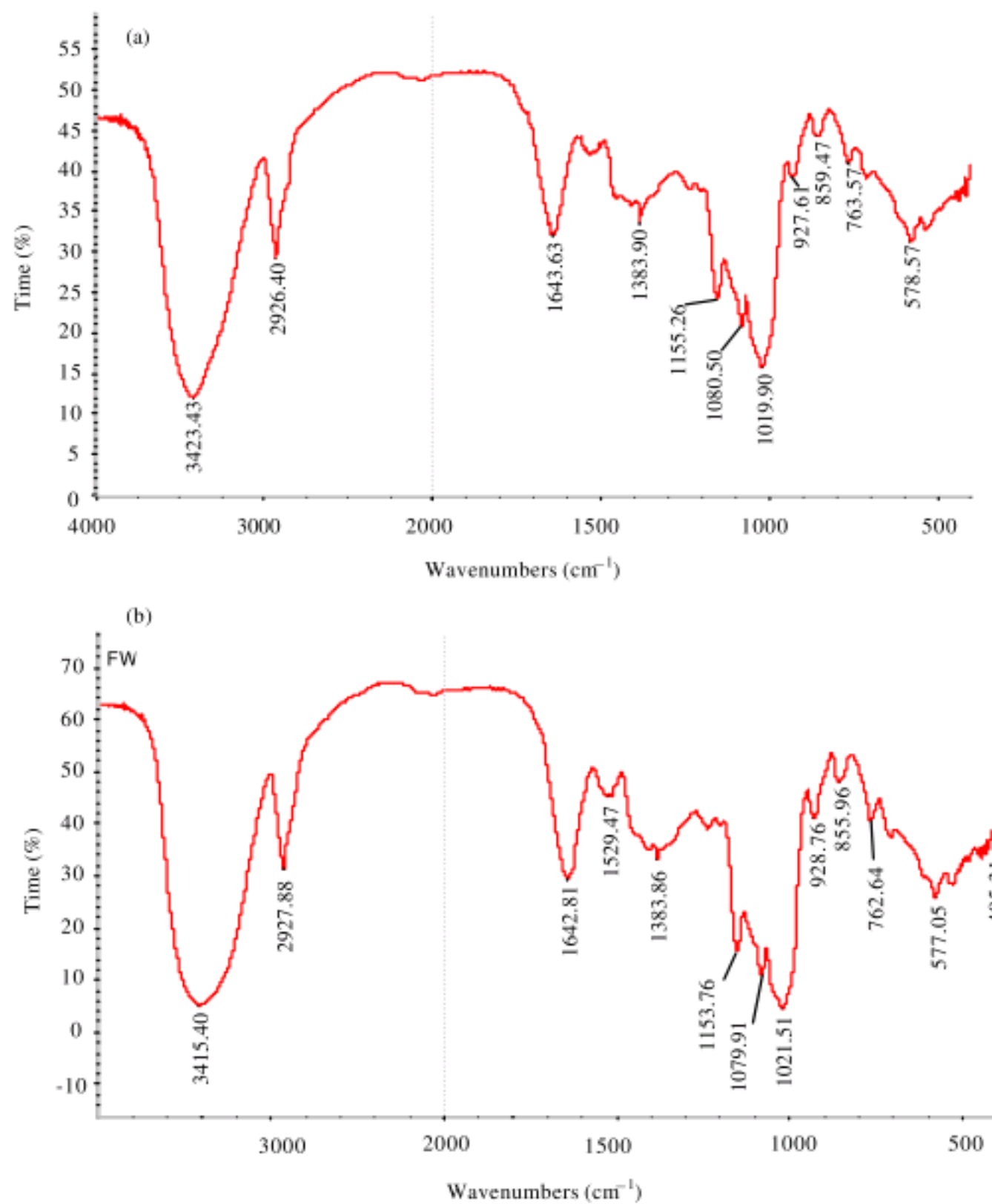


Fig. 3: (a) Infrared spectra for defatted foxtail millet flour white, (b) Infrared spectra for defatted foxtail millet flour yellow

print. By comparing spectra, particularly in the finger print region, it is often possible to tell whether or not the two compounds are identical. The interpretation of the IR as observed shows that there exists a very strong peak, for DFMTW at the ( $3423.43\text{ cm}^{-1}$ ); denoting the likely presence of O-H. This was also observed for DFMTY, but in the region of ( $3415.40\text{ cm}^{-1}$ ); this peak was not as broad as DFMTW. At the right side of the peak, C-H compound was observed to be likely present in both defatted foxtail millet flour (Fig. 3a, b) at the region of ( $2926.40\text{ cm}^{-1}$ ) for the DFMTW and ( $2927.88$ ) For the DFMTY. Within the finger print region ( $1600\text{-}600\text{ cm}^{-1}$ ) DFMTW was observed to have peaks that depicted the presence of Amide I band ( $1643.63\text{ cm}^{-1}$ ) and amide II band ( $1383.90\text{ cm}^{-1}$ ). The difference observed shows that, though the two samples possessed almost the same stretching and bending vibrations. Virtually all organic molecules are infrared active because radiation in this region of the spectrum corresponds to the energy required to excite the natural vibration frequencies of covalent bonds. This is the phenomenon that takes place in the two samples to give the above peaks in the various regions.

### CONCLUSION

The defatted foxtail millet flour for both varieties contains a moderate amount of protein. Furthermore, DFMTW was more digestible than DFMTY. The essential amino acid pattern of the defatted foxtail millet flour suggests their possible use as a supplementary source to most cereals. The defatted millet flours were found to have a higher concentration of low molecular weight polypeptides. Solubilities of both DFMTW and DFMTY at pH 4.0 were similar. Potassium was high in both varieties. Foam capacity, water and oil holding and bulk density were low in both varieties. Total phenolic assay was also low. It shows some differences in the IR analysis. Defatted foxtail millet flour could be used in food formulation with less fear of retrogradation. Further studies on the changes that take place during the traditional processing of defatted foxtail millet are required.

### ACKNOWLEDGMENTS

This research was financially supported by Governments of Sierra Leone and Peoples Republic of China; the authors wish to thank the two Governments.

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