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Starch Functional Properties and Resistant Starch from Foxtail Millet [*Setaria italica* (L.) P. Beauv] Species

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Abstract: The study evaluated physicochemical and content of Resistant Starch (RS) of starches isolated from defatted white foxtail millet and yellow foxtail millet by alkaline extraction. The characteristic of the isolated starches were compared with those of commercial corn starch (CCS). The extraction yielded 42.10% and 39.29% (dry basis) of starch respectively, for white and yellow foxtail millets. The amylose content was highest (35.80mg g^{-1}) in the Yellow Foxtail Starch (YFS) followed by White Foxtail Starch (WFS) which recorded 34.92mg g^{-1} and CCS (33.98mg g^{-1}) with significant difference ($p<0.05$). The amylopectin was found in trace amounts for both WFS and YFS, whereas highest value of carbohydrate was found in CCS (98.59 mg g^{-1}) followed by WFS (98.03mg g^{-1}) and YFS (82.79mg g^{-1}). Microscopic examination showed that all starch granules had sizes ranging from 20 to 50 μm with variable irregular shapes. Millet starch indicated highest degree of syneresis and gel consistency. Swelling and solubility increased as temperature increased from 60 to 95°C. Pasting viscosities and maximum peaks were CCS (75.34°C, 3307cP), WFS (76.09°C, 3321cP) and YFS (76.10°C, 3322cP). The RS in raw defatted and hot boiled starches were (15.77-9.17%) in CCS, (13.35-7.46%) WFS and (14.56-8.24%) YFS, respectively and showed a significant difference ($p<0.05$) among samples.

Key words: Foxtail millet, starch, functional properties, resistant starch, starch digestibility

INTRODUCTION

Starch has extensive commercial utilization as a raw material in many areas including textile, food and pharmaceutical and paper industries among others where biopolymers are widely used (Muir and O'Dea, 1992). It is used in both native and modified form, based on its functional properties such as viscosity, past clarity, gel consistency, degree of syneresis and shape and size of the granules. However, some investigations have observed that considerable amount of food rich in carbohydrates, possessed variable quantities of starches that cannot be digested in the small intestine and passed into the colon (Englyst and Cummings, 1987; Singh and Ali, 2006).

According to *in vitro* digestibility, dietary starches can be grouped into three main fractions: Rapidly Digestible (RDS), Slowly Digestible (SDS) and Resistant Starch (RS). Physiologically, SDS, RS, or the combination of the both could exert significant influence on human health, especially glucose metabolism, management of chronic diet related diseases, mental performance and weight control (Englyst *et al*., 1992). The RS fraction in food may resist digestion and absorption in the small intestine for many reasons. First, starch granules present in whole foods are physically inaccessible to digestive enzymes and become accessible if the food is finely ground; second, the crystalline pattern of the starch granules, dictates susceptibility of the starch to enzymatic digestion (Englyst and Cummings, 1990; Boboi *et al*., 2007).

In resisting digestion in the small intestine, RS, like dietary fiber, becomes available as substrate for fermentation by anaerobic bacteria in the colon (Boboi *et al*., 2007). Therefore, in the large intestine it may share many of the characteristics and health benefits currently attributed to dietary fiber such as diabetes (Elleuch *et al*., 2011), cardiovascular disease and bowel cancer (Boboi *et al*., 2007).

Foxtail millet is dominantly cultivated in Asia especially in China and it has been suggested that China might be the centre of its origin (Jiaju and Yuzhi, 1993). It has a great importance in the neolithic culture in China. Apart from its wide usage as gruel or soup for nourishing pregnant and lactating women, the crop was also used

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as a diet therapy for treating diseases in northern part of China (Li, 1986). The main compositions of foxtail millet grain are starch, protein and lipids, with a small amount of free sugar and non-starch. Though the starch granules in foxtail millet are generally spherical, polygonal forms have been found (Kumari and Thayumanavan, 1998). The amylose and amylopectin contents in the starch depend on the type of foxtail millet: waxy (high in amylopectin), normal (low amylose) or non-waxy (high amylose) (Nakayama *et al*., 1998). In normal foxtail millet, the amylose content can be as high as 33% (Malleshi *et al*., 1986).

Objective of this study is to evaluate the physicochemical properties, pasting behavior and functional characteristics of isolated starches from both White Foxtail Starch (WFS) and Yellow Foxtail Starch (YFS) as well as their resistant starches by *in vitro* enzymatic method and compared to those of the Commercial Corn Starch (CCS). The understanding of these properties could be crucial in utilizing the foxtail millet for the development of composite blends from locally produced small-scale industry level as value-added products.

MATERIALS AND METHODS

Sample: Samples were prepared according to our previous report (Bangoura *et al*., 2011). The corn flour was purchased from Wuxi local market and corn starch was supplied by Quingdao Shengda Commercial & Trade Co.,Ltd, China. Pancreas α-amylase (CAS:9000-90-2), amyloglucosidase (CAS:9032-08-0), amylose (CAS:9005-82-7) and amylopectin (CAS:9037-22-3) were purchased from (Sigma-Aldrich Co.,Ltd, China). All other chemicals and solvents were the highest commercial grade and were used without further purification.

Starch isolation procedure: Starch was isolated from both defatted flours, using the method previously described by Schierbaum *et al*. (1991) with minor changes. Millet flour (100 g) was suspended in 350 mL tap water and 350 mL 0.5% NaOH solution was added with stirring, which was continued for 1 h. The alkaline slurry was centrifuged and the sediment was washed with 350 mL tap water for 30 min. After centrifugation, it was washed again with 160 mL tap water for 15 min. The sediment was suspended in 80 mL tap water, stirred for 10 min before being centrifuged, neutralized with 1.0M HCl and centrifuged again. The upper grayish layer was removed and the white starch was suspended in 100 mL tap water and passed through a 300-mesh sieve with additional tap water (300 mL). The washings were centrifuged (3000 x g 10 min at 20°C) and the resultant starch was air-dried overnight and gently ground to pass through a 250-mesh sieve.

Chemical composition of starches: The CCS, WFS and YFS were analyzed for protein, moisture and ash using the methods described by James (1995). The carbohydrate, amylose and amylopectin were analyzed with the methods cited by Sadasivam and Manikam (1992).

Degree of syneresis and gel consistencies: The degree of syneresis of starch gels was determined from the volume of water (mL) separated from 30 mL of starch gels of 2, 4 and 6% starch solutions and storage at 5°C for 12 h as described by Yang *et al*. (1980; 1984). The gel consistencies were measured according to the method previously described by Radley (1976). Briefly, 2 ml from each concentration of 4, 5 and 6% starch solutions were heated in 15 x 150 mm test tubes for 5 min at 95°C. After storage for 30 min at room temperature (25±1°C), test tubes were placed horizontally on a graph sheet and gel spread (Length) was measured.

Shape and size of starch granules: Size and shape of isolated starches granules were examined using a light microscope (Fei Quanta-200, China). Starch granules were stained with 0.1% iodine solution. For this, 100 mg iodine was mixed in 100 mL of 0.1% potassium iodide solution. Starch granule sizes were determined with an eyepiece micrometer.

Swelling power and solubility pattern: The swelling power and solubility of starch fractions were determined according to the methods described by Tester and Morrison (1990). Sample (0.2 g) was mixed with 10 mL water in 15 mL tarred screw cap tube and incubated in a thermostatically controlled water bath at 90°C. The suspension was stirred intermittently over 30 min to keep the starch granules suspended. The tubes were rapidly cooled to 20°C. The cooled paste was centrifuged at (2200 g x 15 min) to separate the gel and supernatant. The supernatant was immediately recovered and kept for subsequent analysis of solubility pattern. The weight of the swollen sediment was determined. Supernatant was poured into a tarred evaporating dish and put in air oven at 100°C for 4 h. Water solubility index was determined from the amount of dried solids recovered by evaporating the supernatant and was expressed as gram dried solids per gram of sample. Using formulas (1) and (2) for calculation as shown below:

$$\text{Solubility (\%)} = \frac{\text{Residue weight in (g) x Water weight in (g)}}{\text{Aliquot volume in (mL) x Sample weight in (g)}} \text{ x } 100 \quad (1)$$

$$\text{SP (\%)} = \frac{\text{Sedimented starch weight in (g)}}{\text{Sample weight in (g) x } 100 - \text{\% of soluble in (dry basis)}} \text{ x } 100 \quad (2)$$

Rapid Visco Analysis (RVA) pasting profiles: The pasting behavior of the starches is very important for their characterization and applications. A Rapid Visco

Analyzer (RVA-TECMASTER, Newport Scientific Pty. Ltd., Australia) with thermocline windows software was used to evaluate the pasting properties of the starches. Viscogram profile/pasting curves show the relationship between time, viscosity and temperature during cooking processes.

Resistant Starch (RS): The method previously described by Champ *et al.* (1999) and approved by McCleary *et al.* (2002). Briefly, 100 mg of sample was weighed into test tube followed by addition of 10 mL of pancreatic α-amylase solution and mixed thoroughly after 0.1M tris-maleat buffer (Calcium Chloride 4mM) was added. The tubes were incubated in water bath at 37°C for 16 h before being added 40mL EtOH and left to stand for 1 h, then centrifuge at (2500 g x 15 min). The residues were washed with 80% EtOH before being dried at 60°C in vacuum. Then 1.56 mL water and 1.5 mL 4M KOH were added and mixed for 0.5 h at room temperature (25-37°C) and add 12 mL of water. An aliquot 1.5mL was dispersed approximately in 0.65 mL 2M acetic acid (pH 4.5) and 0.1 mL amyloglucosidase (20/0.1 mL 0.1M Na acetate buffer pH 4.5) according to the pH in small intestine and incubated in shaking water bath at 65°C for 90 min. RS was processed using glucose oxidase assay, with the methods cited by Sadasivam and Manikam (1992). The principle of this method involved *in vitro* RS as the starch, which is not hydrolyzed by incubation with α-amylase. Amyloglucosidase was added to avoid inhibition by products of amylase digestion. Hydrolysis products were extracted with ethanol and discarded. The RS was solubilized with amyloglucosidase.

Kinetics digestibility of starch: *In vitro* kinetics of starch digestion was determined according to Goni *et al.* (1996). Defatted millet samples (50 mg) were prepared in 30 ml flasks 10 ml HCl-KCl buffer (pH 1.5) was added and the samples were homogenized for 2 min. A solution (0.2 mL) containing 1 mg of pepsin from porcine gastric mucosa in 10 mL HCl-KCl buffer (pH 1.5) was added to each sample and incubated at 40°C for 60 min in a shaking water bath. The digest was diluted to 25 mL by adding 15 mL Tris-maleate buffer, the pH being adjusted to 6.9 by use of a voltmeter and addition of 2M HCA or 0.5M NaOH as necessary. Starch hydrolysis was initiated by addition of 5 mL of Tris-maleate buffer containing 2.6 IU of porcine pancreatic alpha-amylase. The reaction mixture was incubated in a shaking water bath at 37°C with moderate agitation. Samples (0.1 mL) were taken from each flask every 30 min from 0 to 3 h. The α-amylase inactivated immediately by holding the flasks in boiling water bath for 5 min. Sodium acetate buffer (1 mL 0.4 M, pH 4.75) was added and the residual starch digested to glucose by adding 30 mL amyloglucosidase and incubated at 60°C for 45 min. Glucose concentration was determined by using a glucose oxidase-peroxidase (Sadasivam and Manikam, 1992). The rate of starch digestion was expressed as a percentage of the total starch hydrolyzed at different times (30, 60, 90, 120, 150 and 180 min). Each sample and treatment was analyzed in triplicate. A non-linear model established by Goni *et al.* (1996) was applied to describe the kinetics of starch hydrolysis, following equation (3):

$$C = C_\infty (1 - e^{kt}) \quad (3)$$

Where: C is the percentage of hydrolyzed starch at time t, C_∞ is the percentage of hydrolyzed starch after 180 min, k is the kinetic constant and t is the time (min).

The parameters, C_∞ and k were estimated for each sample in each treatment based on the data obtained from the *in vitro* hydrolysis procedure. The area under the hydrolysis curve (AUC), was calculated using the equation (4):

$$AUC = C_\infty (t_f - t_0) - (C_\infty / k)[1 - \exp 0(t_f - t_0)] \quad (4)$$

Where: C_∞ is the percentage of hydrolyzed starch after 180 min, t_f is the final time (180 min), t_0 is the initial time (0 min) and k is the kinetic constant.

The Hydrolysis Index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample. The Frei *et al.* (2003) equation (5) was used to calculate the Estimate Glycemic Score (EGS):

$$EGS = 39.71 + (0.549\ HI) \quad (5)$$

Statistical analysis: One-way Analysis of Variance (ANOVA) was carried out on each of the variables and the Least Significant Difference (LSD). Test at α level of 0.05 was performed using SAS software (SAS 8.1 for Windows, SAS Inc., Cary, NC, USA) to compare the differences between treatment means. Results were expressed as the means±standard deviation of three separate determinations.

RESULTS AND DISCUSSION

Chemical composition: The proximate composition of isolated millet starches and that of the commercial corn is shown in Table 1. The yields of extracted WFS (42.10%) and YFS (39.27) starches were high. Significant difference ($p<0.05$) was found between both starch yields and this is in good agreement to those reported by Baafi and Safo-Kantanka (2008), who reported yield of the millets' starch to be considerably higher than those of other minor cereals and legumes. Moisture contents were 5.57% for YFS, 5.21% for WFS and 2.0% for CCS, while the values for ash were 0.72%,

Table 1: Proximate analysis of foxtail millet and commercial corn starches[d]

	Foxtail millet and commercial corn starches		
Nutrient	White foxtail	Yellow foxtail	CCS
Yield (%)	42.10±0.13[a]	39.27±0.23[b]	ND
Moisture (%)	5.21±0.05[c]	5.67±0.08[c]	2.00±0.02[a]
Ash (%)	0.72±0.01[b]	1.00±0.17[a]	0.65±0.03[c]
Protein (%)	1.54±0.07[a]	1.04±0.18[ba]	0.81±0.08[cb]
Carbohydrate (mg g^{-1})	98.03±0.37[ab]	82.79±0.53[c]	98.59±0.61[a]
Amylose (mg g^{-1})	34.92±0.11[b]	35.80±0.21[a]	33.98±0.27[c]
Amylopectin (mg g^{-1})	Trace	Trace	ND

[d]Means of triplicates, values in the same rows with different letters are significantly different ($p<0.05$). ND: Not Determined

Table 2: Degree of syneresis and gel consistency measurement[d]

	Syneresis			Gel consistency		
Starch	2%	4%	6%	4%	5%	6%
CCS	17.26±0.3[a] (75)	12.20±0.2[a] (73.2)	10.89±0.1[c] (70)	5.35±0.1[b]	4.28±0.2[c]	3.12±0.1[c]
WFS	17.21±0.6[ba] (69)	12.15±0.3[ba] (67.01)	11.00±0.2[ba] (66)	6.75±0.2[a]	5.75±0.3[a]	4.25±0.1[ab]
YFS	15.13±0.2[c] (67)	11.55±0.3[c] (66)	11.30±0.8[ab] (65.7)	6.25±0.1[a]	5.25±0.1[ab]	4.27±0.2[a]

[d]Means of triplicates, values in the same columns with different letters are significantly different ($p<0.05$).
[e]Values in parentheses show amount of the freed water

1.0% and 0.65% respectively. A subsequent variation of these components was observed with significant difference ($p<0.05$) among the starch samples confirming previous report (Stahl *et al*., 2006). Protein was estimated to be lower in CCS (0.81%) when compared to those of both WFS (1.54%) and YFS (1.04%) foxtail. The CCS (98.59%) had high value of carbohydrate followed by WFS (98.03) and YFS (82.79%). This is in good agreement to the results of Stahl *et al*. (2006). YFS (35.80mg g^{-1}) showed high value of amylose followed by WFS (34.92 mg g^{-1}), while CCS (33.98 mg g^{-1}) had low value of this component. These results exceeded those reported by Fujita *et al*. (1995) and Kim *et al*. (2009), whereas amylopectin showed only the trace in both white and yellow foxtail starches. Furthermore, these nutrients in the starch is a simple convenient way of illustrating its purity, lower contents of other components (protein, ash, etc.) are highly desirable for swelling and pasting abilities. Thus, high contents of other components such as fat and/or protein in starch could influence swelling capacity and pasting behavior.

Degree of syneresis and the gel consistencies: Syneresis and gel consistency measurements of different starches are shown in Table 2. Susceptibility to syneresis is an important property of starches, characterized by water leaking from pastes during cooling. In the freeze-thaw cycle, it was observed that more water was freed from the starch samples. In CCS, it varied from 67 to 75% in relation to the initial weigh, while gel consistency decreased dramatically from 4 to 6% (5.35, 4.28 and 3.12 mm). For WFS it varied from 69 to 66% and gels were (6.75, 5.75 and 4.25 mm) and for YFS it was from 67 to 65.7% the gels were (6.25, 5.25 and 4.27 mm) of diameter, respectively. Therefore, no significant difference ($p<0.05$) was observed between CCS and WFS values of gel concentration in syneresis. YFS showed low freed water and gel and gel consistency failed in CCS against WFS and YFS. Remarkably, CCS, WFS and YFS, after storage at 5°C the syneresis increased in that order, whereas gel consistency showed the reverse (WFS>YFS>CCS) after cooling for 35 min. However, syneresis of starch pastes at 5°C was dependent of time of storage with high water loss being observed in all the starch samples. The prevented past retrogradation in CCS was observed by less consistency of starch paste after heating at 30 min storage. This finding related to the pastes clarity assays and indicated that WFS and YFS starches are also less prone to retrogradation than CCS sample. These remarks were similar to those reported by Stahl *et al*. (2006), when they studied the characteristics of different native starches. Syneresis and gel consistency of all starch gel concentrations were found to be high, except for CCS, which had low values of gel consistency and significantly different ($p<0.05$). This behavior probably related to high amylose content in WFS and YFS samples. No significant difference was remarked for 2% and 4% of starch solutions in syneresis between CCS and WFS samples, while WFS and YFS starches tend to give similar values.

Swelling power and solubility pattern: The swelling power and solubility of starch granules showed a great evidence of interaction on the starch chains between the amorphous and crystalline regions. When starch is subjected to heating in excess water, there is a relaxation of the crystalline structure and the groups of amylose and amylopectin associate with water molecules through hydrogen bonding. This causes an increase in the swelling power and in the solubility of the

Table 3: Swelling power and solubility (%) of CCS, WFS and YFS

	Temperature (°C)			
	60	70	80	90
Swelling				
CCS	7.98±0.26[cb]	9.07±0.30[a]	10.36±0.35[c]	11.66±0.52[c]
WFS	8.25±0.29[a]	9.63±0.29[a]	11.00±0.33[a]	12.15±0.57[ab]
YFS	8.03±0.43[ba]	9.45±0.45[a]	10.80±0.51[ab]	12.38±0.38[ab]
Solubility				
CCS	16.64±0.62[c]	19.60±0.72[c]	22.66±0.82[c]	26.62±0.93[c]
WFS	17.78±0.66[ab]	20.74±0.76[ab]	23.54±0.55[ab]	26.67±0.97[ab]
YFS	17.66±0.41[ab]	20.70±0.72[ab]	23.27±0.42[ab]	26.62±0.93[ab]

[d]Means of triplicates, values in the same columns with different letters are significantly different ($p<0.05$)

granules (Hoover, 2001). In the present evaluation, CCS showed low swelling and solubility with increasing temperature as shown in Table 3. The swelling power increases with the increase in temperature. According to Hashim *et al.* (1992), during the gelatinization, at lower temperature the starch granule has limited swelling in only small amount of solubilized carbohydrate, whereas at 90°C there is an increase in the swelling power and a large amount of carbohydrate leaks from the granular structure. The results of swelling power for CCS samples had lower values than those of WFS and YFS samples, probably due to the procedure of extraction, which turned the granules weaker and easier to be disrupted. That easily exemplified the swelling of the native starches.

The native WFS and YFS starch showed higher swelling power when compared to the CCS samples, despite the fact that the value (12.38 times at 90°C) was much lower than that found in the literature (1159 times at 90°C) (Leach *et al.*, 1959).

The solubility increased with the increasing temperature as mentioned by Paterson *et al.* (1994). Because the CCS, WFS and YFS starch pastes presented high transparency, there was difficulty in identifying the separation of the two phases after centrifugation and some of the results were lost.

Shape and size of starch granules: Microscopic examination of CCS, WFS and YFS starch granules are shown in Fig. 1. This observation showed that most of the starch granules had irregular shapes, which varied from oval, round to bean-shaped Fig. 1A, B and C, respectively. Among starches granules, CCS and YFS had similar size and showed little swelling at room temperature as observed under optical microscopy with same granule sizes (20 µm, 5.0 KV) while WFS presented a light color and the granules were mostly close to each other, tend to do not have faint hilmus. Besides, starch granules of YFS were similar to those of CCS with rounded shape and centric hilmus Fig. 1A and C. However, under optical microscopy at 5.0 KV, the size of all granule samples were 50 µm and the pores showed fewer fissures on starch granules. Moreover, a few faint hilmus and very weak centric polarization were expected (Fig 1D, E and F).

Structural change induced by changing the length of optical microscopy for understanding the astuteness of the starch granules according to the starch clarities (Fig. 1E, 1F and 1G). Generally, all the native starch granules were birefringent and showed the characteristics "Maltese cross" pattern under polarized light (Fig. 1G, 1H and 1I). The characteristics of the WFS and YFS granules were similar with considerable faint hilmus, especially more observed in CCS granules when compared to those of the WFS and YFS granules. This was a least similar to those reported by Fujita *et al.* (1995) and Kim *et al.* (2009).

RVA Pasting profile: The pasting properties of both extracted foxtail millets WFS, YFS and CCS are presented in Table 4 and Fig. 2. The rapid Visco-analyzer was instrumental to perform the pasting behaviors of isolated and commercial corn starches. The millet starches showed high peak viscosities (3321cP and 3322cP), due to the high amylose content in their starches when compared to that of CCS (3307cP). No relation was observed between swelling power and RVA pasting curves (Lorenz and Hinze, 1976). Therefore, both swelling power and amylograph viscosity are usually determined when functional characteristics of starches are studied. The viscosities of all starch samples at temperature ranged from 75.34 to 76.10°C were considerably high and no significant difference was observed among them. Holding strength (2122cP and 2123cP) and final viscosity (3745cP and 3747cP) of both extracted starches showed high values to those of the CCS (1617cP and 3305cP), respectively. While breakdown and setback (1690cP and 2130cP) had high values in CCS than those of YFS (1299cP and 1199cP) and WFS (1290-1197cP). The RVA viscosity of CCS was much lower in WFS and YFS. The lower breakdown (difference between peak and viscosity strength) imply higher hot paste stability (resistance to shear thinning during cooking) for CCS starch. The results indicated that the RVA viscosities for WFS and YFS were comparatively higher to that of CCS, but pasted starch lost viscosity during holding period and this may be attributed to the fragmentation and solubilization of the swollen granules (Lorenz and Hinze, 1976).

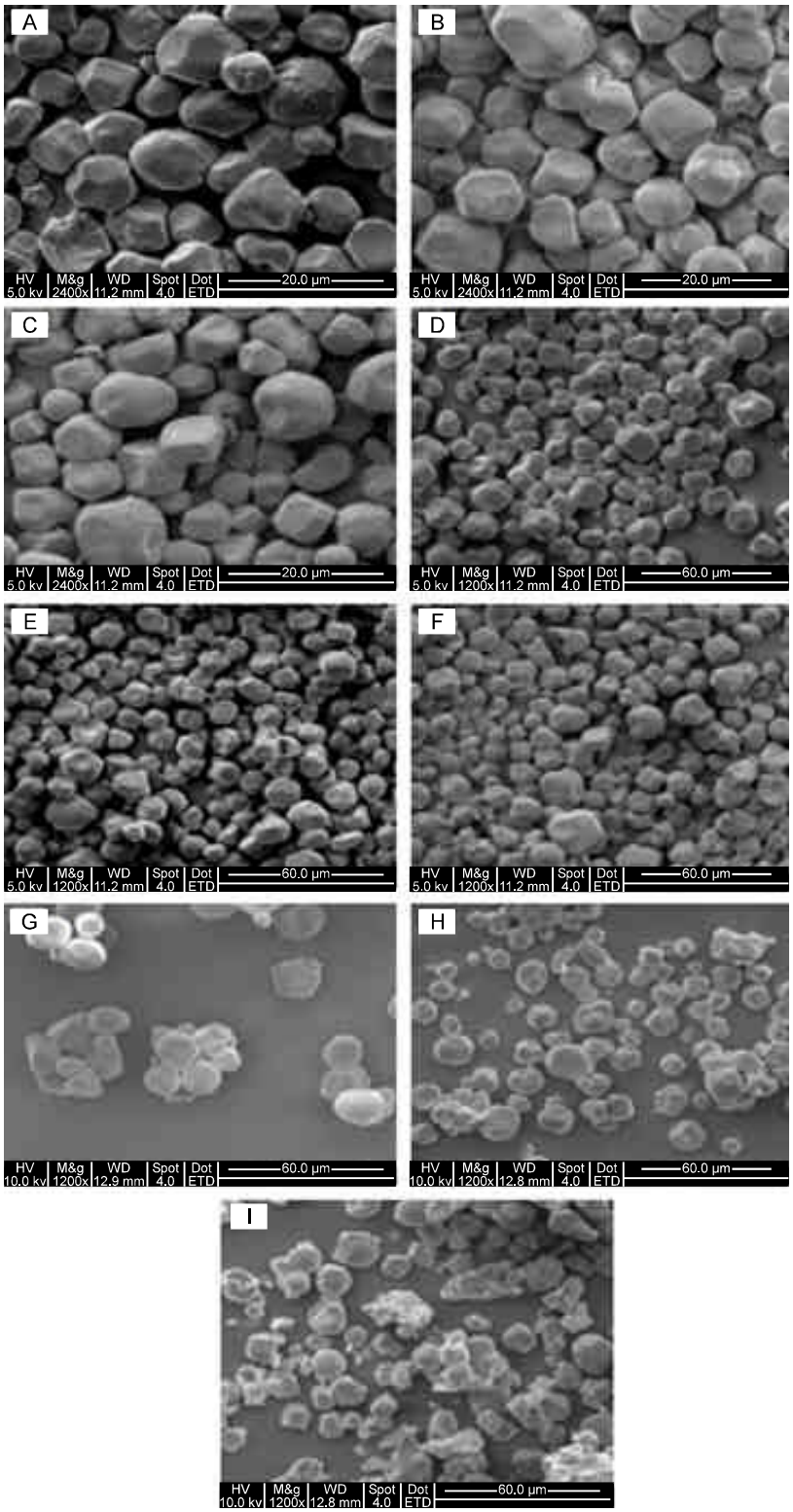

Fig. 1 (A, B, C, D, E, F, G, H and I):Scanning electron micrograph of CSS-A, WFS-B and YFS-C at (20 µm, 5 kv), CSS-D, WFS-E and YFS-F at (50 µm, 5 kv) and CCS-G, WFS-H and YFS-I at (50 µm, 10 kv)

Table 4: Pasting properties of WFS, YFS and CCS starches

Starch (10%)	Pasting time (min)	Pasting temperature (°C)	Viscosity (cP) Peak viscosity	Holding strength	Final viscosity	Breakdown	Setback
CCS	4.6	75.34	3307	1617	3305	1690	2130
WFS	4.7	76.09	3321	2122	3745	1290	1197
YFS	4.7	76.10	3322	2123	3747	1299	1199

Table 5: Total starch and resistant starch content in defatted and boiled foxtail millet[d]

Defatted sample	TS	RS defatted	RS boiled
CCF	ND	15.77±0.13[a]	9.17±0.04[a]
WFF	57.57±0.30[a]	13.35±0.09[c]	7.46±0.12[c]
YFF	52.44±0.29[b]	14.56±0.12[b]	8.24±0.13[b]

[d]Means of triplicates, values in the same columns with different letters are significantly different ($p<0.05$)

Table 6: Model parameters, Hydrolysis (HI) and Estimated Glycemic Score (EGS) of fresh and cooked millet starches[d]

Sample	Treatment	C_∞	k	HI	EGS
CCS					
	Fresh	70.01±0.3[c]	0.211	102.3±0.02[bc]	92.27±0.17[cb]
	Cooked	68.45±010[b]	0.097	98.56±0.12[b]	89.12±0.15[b]
WFS					
	Fresh	71.12±0.6[b]	0.224	106.02±0.41[b]	98.57±0.15[ba]
	Cooked	68.27±0.2[cb]	0.093	98.49±0.22[cb]	88.99±0.21[cb]
YFS					
	Fresh	72.23±0.4[a]	0.238	112.31±0.13[a]	100.04±0.11[a]
	Cooked	69.18±010[a]	0.099	99.39±0.18[a]	93.76±0.19[a]

[d]Means of triplicates, values in the same columns with different letters are significantly different ($p<0.05$)

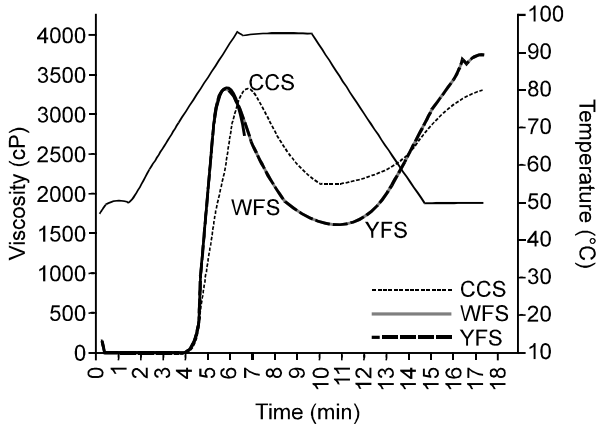

Fig. 2: RVA Viscogram of CCS, WFS and YFS starches

Resistant Starch (RS): The RS total was found to be considerable high in both foxtail millet species and significantly different ($p<0.05$). In this study, the RS in raw defatted and boiled (white and yellow) foxtail millets was evaluated, using the method newly described by Champ *et al.* (1999) and McCleary *et al.* (2002) for a specific determination of RS in foods. RS values were variable and low when determined by the three methods (RDS, SDS and RS) and these results were not shown in this investigation.

The RS value in the both defatted and hot boiled millets are presented in Table 5.

In defatted millets, the RS values were significantly high and different ($p<0.05$) and lower when compared to defatted corn. However, RS enrichment in both white and yellow foxtail millets had low values in boiled samples, whereas that the RS was obviously increased in boiled corn. These results were within the range of those reported in previous studies, using other methods (Kosoko *et al.*, 2011). This behavior has significant implications for use of RS in food formulations to help persons with diabetes normalize glucose pressure. There is evidence that slowly digested and absorbed carbohydrates are favorable for the dietary management of metabolic disorders such as diabetes and hyperlipidemia (Pongjata *et al.*, 2009). The starch fraction including RS fermented by microflora can produce short-chain fatty acids such as acetic, propionic and butytic acid that are greatly helpful in preventing colonic diseases (Noor Aziah *et al.*, 2011).

Kinetic model of starch digestibility: The kinetic parameters that described the hydrophilic process of starch digestion were obtained (Table 6). C_∞ represent the equilibrium concentration reached after 180 min of hydrolysis and the constant "k" standards for the kinetic (digestibility) constant (i.e, intrinsic susceptibility of starch in the product to digest). The kinetic constant from YFS was significantly different to those of CCS and YFS, suggesting that differences in digestibilities were due the innate properties of their starches. On the other hand, both WFS and CCS gave different kinetic constant. Therefore, the differences in the digestibility among these starches were probably due to extrinsic factors (Ezeogu *et al.*, 2005). The HI, that represents the

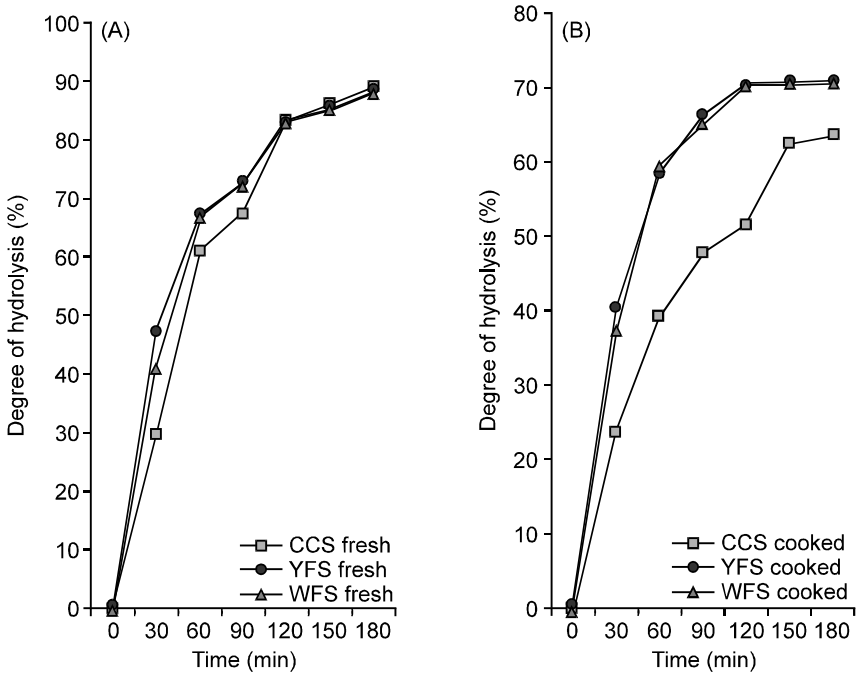

Fig. 3: *In vitro* starches hydrolysis of CCS, WFS and YFS fresh (A) and cooked (B) starch samples

proportion of the starch that theoretically digestible (under the conditions of study) (Ezeogu *et al*., 2005). Utilizing HI value in the formula (Goni *et al*., 1997), the Estimated Glycemic Score (EGS) for all starch samples was significantly different (Table 6). The highest HI values were reported in fresh and low values in cooked samples. The HI values for both fresh and Cooked were ranged, for YFS (112.31 and 99.39), followed by WFS (106.02 and 98.49) and CCS (102.3 and 98.56), respectively. The retrogradation affects mostly the cooked CCS of its low Amylose Content (AC) and YFS has fairly affected, whereas CCS and WFS showed a similar HI values. Therefore, no EGS data was available for foxtail millet starch for comparison.

From Fig. 3 the extend time of starch hydrolysis for fresh A and cooked B of the starch samples showed a slight variation according to their Amylose Content (AC). Corresponding to the RS results, the rate and extend of starch hydrolysis were different between the fresh and cooked starch samples. Remarkably, the samples, which had high RS, showed high resistant to hydrolyze and their hydrolysis completed in high degree. This was primarily concerned with the both WFS and YFS. CCS with its low AC hydrolyzed slowly to reach completion after 120 min, whereas in the cooked samples reached times of hydrolysis are differed due to degradation of amylose during cooking.

Therefore, in cooked starch samples for both YFS and WFS showed similar increase curve and hydrolysis completed in high degree (70.83% and 70.55%), respectively after 90 min, whereas CCS hydrolyzed slowly and had low degree (63.57%), completed after 120 min. Yang *et al*. (2006) also confirmed this observation. In addition, slow digestible starch play an important role in human physiology.

Conclusion: Current study revealed that foxtail millet starches from two varieties could be classified into group according to the cultivars showing high amylose content and high RS production. The RS was higher in defatted samples whereas the values of the RS were lower in boiled samples which had the order as corn>white>yellow, while the amylose content was in the order of white>yellow>corn.

Starch extraction processing method showed that the inherent chemicals and functional characteristics of both millet starches played an important role in the formation of RS in both defatted and boiled starch samples. The high RS in foxtail millets could differ significantly among species in variable aspect of starch properties such as high amylose, starch granule shape and size, starch paste viscosity and syneresis. The high RS in foxtail millets could significantly differ among the species in different aspects of the starch proprieties such as high amylose, starch granule shape and size, starch paste viscosity and low gelatinization temperature. The EGS values were more considerably high in YFS and WFS than that of CCS due to its low amylose content. Further research and development in these aspects that could be integrated with other current ongoing research activities is necessary.

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