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FUNCTINAL, PASTING AND THERMAL CHARACTERISTICS OF FINGER MILLET AND LITTLE STARCH

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ABSTRACT

Finger millet and little millet starch was isolated and subjected to functional properties (swelling, solubility and water absorption capacities), thermal properties, pasting properties and SEM analysis of the starches were determined. Swelling power the starch samples increased with increase in temperature. Water absorption capacities of little millet starches decreased, but finger millet starch showed higher value with high swelling power. The modified starches showed increase in alkaline water retention. The minimum amylose content was present in little millet (18.8%), while maximum was 24.8% in finger millet. The maximum amylopectin content was present in little millet (25.5%). Finger millet starch exhibited comparatively better transmittance than little millet starch. Finger millet showed a higher Peak Viscosity (2937 cP) and T (2233 cP) than the little millet starch. The highest enthalpy of the gelatinization (Δ Hgel) was in 'finger millet starch'. The highest gelatinization temperature was obtained for 'finger millet starch' (19.3°C) compared too little millet. SEM images resulted that finger millet starch and little millet starch granules were mainly polyhedral in shapes. A positive correlation coefficient was observed between little millet amylase content and finger millet setback viscosity ($p < 0.01$). A positive correlation was observed between finger millet amylopectin and finger millet amylase at 0.05% level.

Keywords: finger millet, little millet, amylase, amylopectin, peak viscosity, starch.

INTRODUCTION

Millets are small-seeded cereals having excellent nutritional quality. They are comparable or superior to some commonly consumed cereals like wheat and rice (Ragaee *et al.* 2006). Despite its superior nutritional quality it has received less attention compared to the major cereals. They are gradually gaining importance in the North American and European countries due to its gluten-free and hypoglycemic property. A few studies have focused on the nutrient quality of pearl millet however documentation on the other types is limited. Millets are also preferred to be decorticated to improve sensory quality and bioavailability of nutrients (Lestienne *et al*. 2005; Shobana and Malleshi 2007).

Starch is often used as a thickener, water binder and emulsion stabilizer and gelling agent. It has a unique property to absorb water, yielding gel, if its suspension is heated. However, the native starches are functionally restricted for food applications because of their structural weakness. Native starch pastes and gels may revert or retrograde back to insoluble form. Hence, modification or processing of starch is necessary to engender their range of functionality. Modification is a process of altering starch structure by affecting the hydrogen bond in a controllable manner to change the form/shape of granule and composition of amylose and amylopectin molecules. Usually, starch modification can be done by several methods such as physical, chemical, enzymatic or genetic transformation (Yiu, *et.al.,* 2008). Acid modification changes the physicochemical properties of starch without destroying its granule structure (Lawal *et.al.,* 2008). During hydrothermal modification, starch properties are modified through controlled application of heat and moisture.

Finger millet is a highly nutritious staple food crop cultivated in many parts of India and Africa (Hood, 1975). Finger millet is nutritionally superior to all other cereals (Sharavathy *et.al.,* 2001 and Arthi *et.al.,* 2003). It is rich in dietary fibre and calcium (Malleshi, 1993 and Premavalli *et.al.,* 2003). In African countries, it is used as an infant and a refreshment food for adults and convalescents. Finger millet is a potential source of commercial native starch exhibiting functional synergism with tapioca. Present investigation was undertaken with an objective to optimize the process conditions for starch isolation from finger millet and also to study the characteristics of native and modified finger millet starch.

Starch contributes greatly to the textural properties of various foods and has many industrial applications as a thickener, colloidal, stabilizer, gelling

agent, bulking agent, water retention agent and adhesive. With increasing industrial demand for starches, there is a need to explore new and alternative sources of starch. Among the cereal starches, much research attention has been given to rice (Takeda *et al.*, 1986; Hizukuri *et al*., 1989), wheat (Takeda *et al*., 1984; Shibanuma *et al*., 1996), barley (Takeda *et al*., 1999; Yoshimoto *et al*., 2000) and maize (Takeda and Preiss, 1993). Keeping in view of the increased applicability of starch in food systems, different cheap and efficient alternative sources of starches with good functional properties are being explored. Pearl millet being an underutilized poor man's food crop can be a cheap alternative source of starch as it has been reported to contain about starch (Freeman and Bocan, 1973). In spite of the fact that the starch in the pearl millet represents 59 to 80% of the endosperm, however, pearl millet starches have been studied less extensively as compared to other conventional sources of cereal and tuber starches. The pearl millet starch granules are slightly smaller than that of corn and sorghum starch granules (Freeman and Bocan, 1973) ranging from 8 to 12µm in diameter. Starches from various Indian cultivars of pearl millet starch show greater variability in amylose content ranging from 18.3 to 24.6% (Singh and Popli, 1973). Hoover *et.al.* (1996) reported pasting temperature of pearl millet starches as 90°C, which was higher than those of wheat (86°C) and corn (81°C) starches.

MATERIALS AND METHODS

STARCH ISOLATION

Finger millet and Little millet grains were obtained from Millet Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India. The seed were hand-sorted to remove wrinkled, moldy seeds and foreign material and then stored in polyethylene bags in the refrigerator (40C±1) until used. Required amount of other food ingredients like whole wheat flour, canola oil, dry yeast, salt, vegetable oil, baking powder, *etc*. was collected from the local Super market of Salem city, cleaned and stored.

ISOLATION OF STARCH

The grain was steeped in 0.2M acetate buffer (pH 6.5) containing 0.01M mercuric chloride for 30 hours at 6 _C which was used to soften the grain and to inhibit amylases. The softened grains were then ground using a Waring blendor and slurried in water before being sieved successively and rapidly through 80 mesh screens. Grinding, slurrying and sieving were repeated until the material left on the sieve was free from starch. Contaminating proteinaceous material in the starch suspension was removed without causing any modification of the starch by shaking the aqueous suspension with 1/8 of its volume of toluene, the protein being denaturated at the water/toluene interface.

FUNCTIONAL PROPERTIES OF STARCH

The functional properties such as water binding capacity (WBC), solubility and swelling power, transmittance and viscosity were determined using standard methods. WBC (%) of starch was determined dispersing 5 g of starch in 75 ml of water following the method of Yamazaki (1953) as modified by Medcalf and Gilles (1976). The swelling power and water solubility of pearl millet starch were measured at temperature ranging from 60 to 90°C using method given by Schoch (1964) with slight modification and the results were expressed as g/g of dry starch. Light transmittance (%) of the isolated starch samples was measured as described by Craig *et al.* (1989) using an aqueous suspension (2%) of starch heated in a water bath to 90°C for 1h with constant stirring.

PASTING PROPERTIES OF STARCH

Pasting properties of starch were evaluated with Rapid Visco-Analyzer (RVA Starch Master, Newport Scientific, Warriewood, Australia). Test profile STDI (Newport Scientific Method1, Version 5, 1997) was used for determination of pasting characteristics. A programmed heating and cooling cycle was used where the starch suspension (6%, w/w) was held at 50°C for 1 min, heated to 95°C at 6°C/min, held at 95°C for 2.7 min before cooling from 95 to 50°C at 6°C/min and holding at 50°C for 2 min. The pasting curve obtained were analyzed using a RVA Starch Master Software setup Tool (SMST) to obtain the characteristic parameters like pasting temperature (Ptemp); peak viscosity (PV, maximum paste viscosity achieved in the heating stage of the profile); hot paste viscosity (HPV, minimum paste viscosity at 95C); cool paste viscosity (CPV, final viscosity at 50°C); breakdown (BD = PV-HPV); set back (SB = CPV-PV), consistency (CS = CPV-HPV), stability ratio (HPV/PV) and set back ratio (CPV/HPV). All measurements were taken in triplicate.

THERMAL PROPERTIES

Differential Scanning Calorimetry (DSC) studies were performed using a DSC (Perkin-Elmer Corp., Norwalk, CT) equipped with a digital DEC-425 thermal analysis data station. The instrument was calibrated using indium and purified, deionised distilled water as standards. Starch sample (3.5 mg, dwb) was weighed directly into aluminium pans, followed by addition of 8 ml of purified, deionised distilled water. The pans were hermetically sealed and allowed to equilibrate at room temperature overnight. A sealed aluminium pan containing 8 ml of purified, deionised distilled water was used as a reference. Samples were heated from 40 to 130°C at a rate of 10°C/min. Enthalpy (ΔH), onset (T), peak (Tp), and conclusion (T) temperatures were computed automatically. The gelatinization temperature range (*R*) and peak height index (PHI) were calculated as (Tc – To) and ΔH/(Tp – Tc) respectively, as described by Krueger *et al.* (1987). After conducting thermal analysis, the samples were stored in a refrigerator at 4°C for 7 days for retrogradation studies. These samples were left at 28 °C for 2 h before analysis. The sample pans containing the starch were reheated at the rate of 10°C/min from 25 to 100°C. The enthalpy of retrogradation was calculated automatically and % retrogradation (% R) was calculated as:

$$\% R = \frac{\text{Enthalpy of retrogodation}}{\text{Enthalpy of gelatinization}} \times 100$$

AMYLOSE/AMYLOPECTIN RATIO

The amylose concentration was calculated using the blue value as defined by Morrison and Laignelet (1983). Amylose content also was determined by highperformance size-exclusion chromatography (HP-SEC) according to the method used by Demeke *et al* (1999), which is based on the debranching of starch using iso-amylase and separation of the fractions obtained. Amylose content was calculated as the ratio of the integrated peak area of the amylose peak and the total peak area that consists of the amylose and the debranched amylopectin peaks.

SCANNING ELECTRON MICROSCOPE (SEM) STUDIES

The physical nature of the starch granules was studied using a scanning electron microscope (JSM – 5300) with an ion sputtering device. The sample under study was viewed at different angles by a suitable rotation device provided in the instrument. Hence, it was possible to get a 3-dimensional picture over a practical magnification range up to 20,000. In the present study, a magnification range between 1000 and 7500 was used. The specimen was mounted on a metal stub for viewing. For starch, which is a non-conducting material, a thin layer (200 Å thick) of metal (usually gold) coating was made to carry off the charge arising from the primary electron beam.

STATISTICAL ANALYSIS

All measurements were replicated a minimum of two times. Data were analyzed using IBM software. Analysis of variance (ANOVA) was used to determine whether starch characteristics differed among lines.

RESULTS AND DISCUSSION

FUNCTIONAL PROPERTIES OF STARCHES ISOLATED FROM RAW MILLET GRAINS

Table 1-Water Binding Capacity (%) and Swelling Power (g/g)

S.no	Raw Millet Starches	Water Binding Capacity (%)	Swelling Power (g/g)	't' value
1.	Finger Millet Starch	358.9±38.1	6.33±0.4	456.581**
2.	Little Millet Starch	318.5±29.4	5.41±0.31	6.591*

** - significant at 0.01%, * - significant at 0.05%

Water binding capacity (WBC) is an important parameter that determines starch use in products. It affects functional properties such as viscosity, which is a very important indicator of bulking and consistency of products (Baafi and Safo, 2007). The values obtained ranged from 318.5% to 358.9%. The WBC for finger millet starch was significantly ($P \leq 0.05$) higher than the little millet starch samples. This difference is occasioned by the available of water binding sites which was very predominating in finger millet starch and this was in agreement with report by Abraham (1993).

Swelling of millet starch was shown in Table 1. Swelling power increased as temperature increased in native and modified starches. This result agrees with the observations of (Gebre and Schmidt, 1996), which indicate that swelling power of onset starch increased as temperature increases. Among the selected millets, finger millet (6.33g/g) showed more swelling power the little millet (5.41g/g). Such phenomenon was reported by Zuluaga *et al.* (2007) and was possibly attributed to additional interactions between starch and other components at this temperature. Swelling power of starch depends on the capacity of starch molecules to hold water through hydrogen bonding and is influenced by a strong micellar network, amylopectin molecular structure and amylose content (Tang *et.al.*, 2005 and Gujska *et.al.*, 1994). The increase in swelling power of finger millet starch observed can be attributed to increase in long chains of amylopectin and decreasing amylose content and is in agreement with reports by Sasaki and Matsuki (1998) and Srichuwong et al. (2005). The reduced swelling power of little millet starch indicates increase in starch crystallinity which restricted the percolation of water within the starch matrices (Hoover and Manuel, 1996).

Table 2- Amylose and Amylopectin content, Light Transmittance (%T) and % Total starch hydrolysed

S:NO	Raw Millet Starch	Amylose (%)	Amylopectin (%)	Light Transmittance (%T at 650nm)	% Total starch hydrolysed at 180 minutes
1.	Finger Millet Starch	24.8	8.0	5.89±0.14	65.8±1.87
2.	Little Millet Starch	18.8	25.5	2.28±0.35	44.9±1.3

Morall and Briggs (1978) reported a 65% decrease in starch content in germinated barley. The decrease in starch content in the grain was due to hydrolysis by native enzymes (α- and β-amylases) during germination resulting in increase in reducing sugars (Dewar *et al.*, 1995). The minimum amylose content was present in little millet (18.8%), while maximum was 24.8% in finger millet. The maximum amylopectin content was present in little millet (25.5%). Paste clarity (% transmittance) for finger millet starch increased with modification. Finger millet starch exhibited comparatively better transmittance than little millet starch. Increase in paste clarity after hydroxypropylation for finger millet and maize starch was reported by Lawal (2009) (finger millet starch) and Liu et al. (1999) respectively. This may be attributed to greater stability of starch structure after modification due to the improvement in inter and intra molecular bonding. This characteristic is particularly useful in foods such as jellies and fruit pastes to get desired consistency.

Table -3 Pasting properties finger millet and little millet

S:no	Raw Millet Starches	Pasting temperature (°C)	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)
1.	Finger Millet Starch	50.2	2937	2233	704	3625	1392
2.	Little Millet Starch	94.55	477	463	14	1255	792

Secondary increase in viscosity (setback) during cooling phase was observed to be minimum for little millet (792 cP). This retrogradation or setback is influenced by various factors viz., amylose content, length of amylose molecules and state of dispersion of amylose chains (Savarin et.al., 1969). The process of gel formation and setback depends on polymer association especially the linear amylose fraction presented in starch molecule (Waldt and Kehoe, 1959). A tendency was noted for waxy-textured potato starch to exhibit low peak paste viscosity and a high degree of setback (Hopkins and Gormley, 2000). Little millet showed lower values for pasting characteristics than other starch samples (Table 3). Finger millet showed a higher Peak Viscosity (2937 cP) and T (2233 cP) than the little millet starch. From these observations it may be concluded that the enzymatic and acidic modification are more effective. It was reported that the low peak viscosity indicates lower water holding capacity for starch (Sekine, 1996).

Amylose content is believed to have a marked influence on the breakdown viscosity (measure of susceptibility of cooked starch granule to disintegration) and the setback viscosity (measure of recrystallization of gelatinized starch during cooling) (Lee *et al*., 1995). Lower level of amylose to reinforce the molecular network within the granules resulted in greater breakdown viscosity. High amylose content has also been suggested as the major factor contributing to the non-existense of a peak, a high stability during heating, and a high setback during cooling (Lii and Chang, 1981, Karim *et al*., 2000).

Table 4 -Thermal Properties finger millet and little millet

S:No	Parameters	Finger Millet Starch	Little Millet Starch
1.	Onset Temperature (T_o) °C	76.6	104.3
2.	Peak Temperature (T_p) °C	82.4	107.2
3.	Conclusion Temperature (T_c) °C	95.9	113.8
4.	Gelatinization Temperature ($T_c - T_o$) °C	19.3	9.5
5.	Enthalpy of Gelatinization (J/g^{-1})	0.643	0.036
6.	Peak Height Index	0.11	0.012

Thermal properties of starches separated from two different varieties are summarized in Table 4. The transition temperatures (To, Tp, and Tc), the range of gelatinization temperature (Tc-To), enthalpies of gelatinization (ΔHgel), and peak height indices (PHI) of starches differed significantly among the varieties. Thermal properties of two isolated starches of finger and little millets showed that the onset temperature was different. The variations in initial gelatinization temperature may be attributed to the differences in amylose content, size, shape, and distribution of starch granules, and to the internal arrangement of starch fractions within the granules. Transition temperatures are influenced by the molecular architecture of the crystalline region, which correspond to the relative ratio of amylose and amylopectin (Noda et al., 1998). DSC parameters showed that the onset temperature (To) of all starches ranged from 76.6 to 104.3 °C, peak temperature (Tp) ranged from 82.4 to 107.2°C, and their gelatinization enthalpy varied from 0.643 to 0.036 J g^{-1}. The highest enthalpy of the gelatinization (Δ Hgel) was in 'finger millet starch'. The higher Δ Hgel of starches suggests that the double helices (formed by the outer branches of adjacent amylopectin chains) that unravel and melt during gelatinization are strongly associated within the granule. The highest gelatinization temperature was obtained for 'finger millet starch' (19.3°C) compared too little millet. The gelatinization enthalpy also reflects the overall measure of crystallinity (quality and quantity of crystallites against amorphous regions) of amylopectin, and is also an indicator of the loss of molecular order within the granules (Tester & Morrison 1990). The peak height index (PHI) refers to the ratio of ΔHgel for gelatinization to the gelatinization temperature range and is a measure of uniformity in gelatinization. The PHI in two millet starches, 'finger millet starch' showed the highest value, indicating that the higher PHI value is attributed to the presence of large size granules (Aggarwal et al., 2004). Paredes-Lopez (1994) reported that low peak viscosity is due to short chain length and to irreversible damage treated with alkaline media. Setback values in nonglutinous proso millet starches were higher than the waxy proso millet starches. It is generally recognized that if viscosity of setback is high, the retrogradation of starch paste would progress rapidly (Leeiarathi et al., 1987). $\Delta H_{reflects}$ the loss of double helical rather than crystalline order (Cooke and Gidley, 1992).

SCANNING ELECTRON MICROSCOPY OF FINGER MILLET AND LITTLE MILLET STARCH

Figure 1 and 2 shows the images of isolated finger millet starch and little millet starch viewed at 2500 x g and 3000 x g magnification viewed under SEM. It is evident that finger millet starch and little millet starch granules were mainly polyhedral in shapes. Their shapes and sizes are in average of 1 to 10µm and these results are in agreement with a previous study by Sodhi and singh (2003). The smooth surface of the granules of these starches indicated the presence of undamaged starch granules. Thus, the shared morphological properties of finger millet starch and little millet isolated starches indicated that the germination process did not affect the overall structure and size of rice starches. Furthermore, it

has been reported that the size of starch granule may affect its physicochemical properties, such as gelatinization, pasting, enzyme susceptibility, crystallinity and solubility (Lindeboom *et al.*, 2004). Variations in the sizes of millet starch granules have been observed for different species.

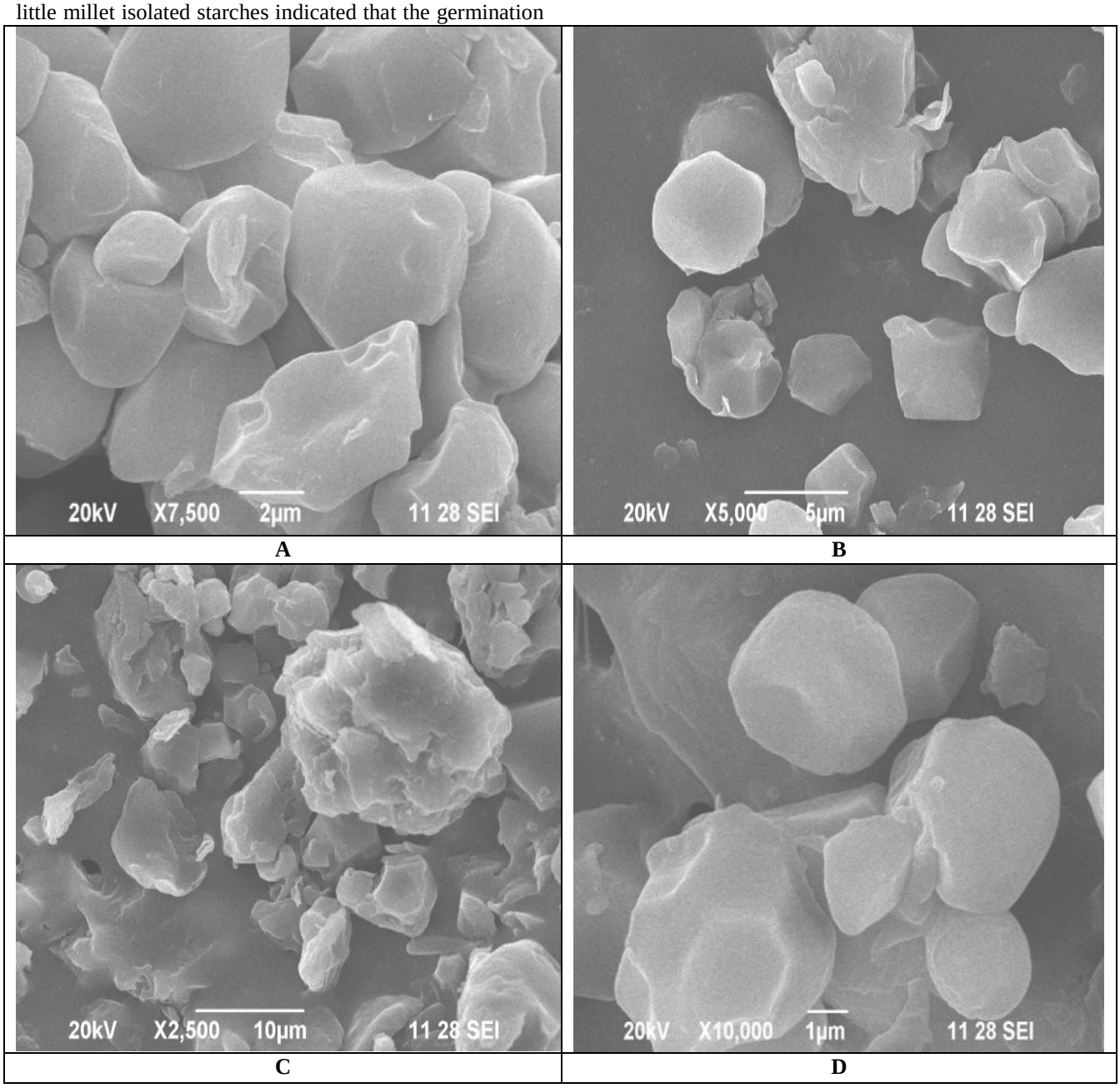
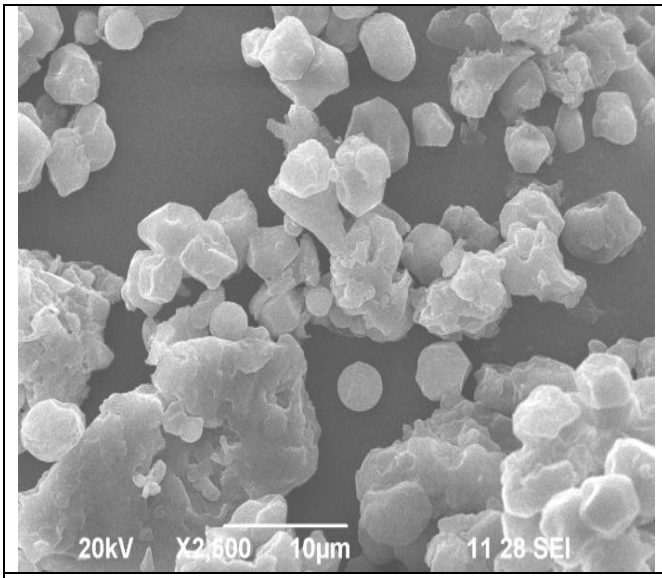


Figure -1 - Scanning electron photomicrographs of finger millet starch

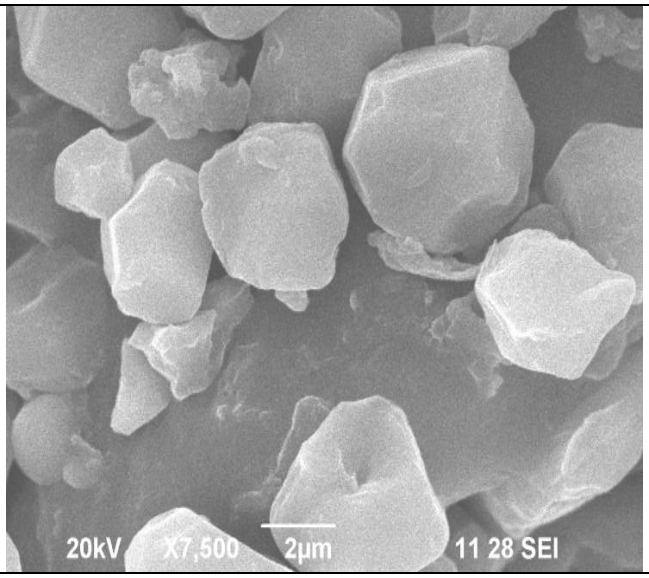
Correlation between amylase, amylopectin, peak viscosity and setback viscosity with finger millet and little millet starch

Correlations								
	FM - peak viscosity	**LM - peak viscosity**	**FM-set back**	**LM - set back**	**FM - amylose**	**LM - amylose**	**FM-amylopectin**	**LM - amylopectin**
FM -peak viscosity	1							
LM -peak viscosity	0.936	1						
Fm-set back	0.164	0.500	1					
Lm -set back	0.936	1.000**	0.500	1				
Fm - amylose	.276	0.596	0.993	0.596	1			
LM - amylose	0.164	0.500	1.000**	0.500	0.993	1		
FM-amylopectin	0.347	0.655	0.982	0.655	0.997*	0.982	1	
LM - amylopectin	0.853	0.982	0.655	0.982	0.737	0.655	0.786	1
**. Correlation is significant at the 0.01 level (2-tailed).								
*. Correlation is significant at the 0.05 level (2-tailed).								

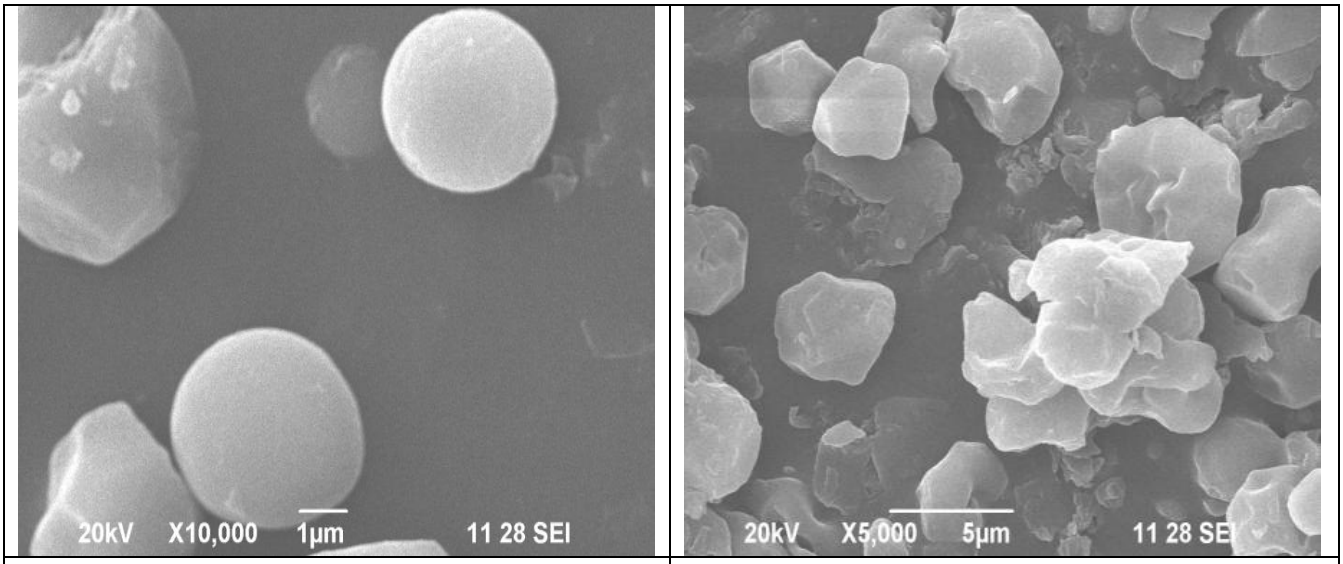
Correlation coefficients were determined to examine the relationships between relative amylase, amylopectin, peak viscosity and setback viscosity (SBV) in two millet starches examined in this study. A highly positive correlation was obtained between little millet setback viscosity and little millet peak viscosity ($p < 0.01$). A positive correlation coefficient was observed between little millet amylase content and finger millet setback viscosity ($p < 0.01$). A positive correlation was observed between finger millet amylopectin and finger millet amylase at 0.05% level. The peak time and peak viscosity are indicative of the water-binding capacity of the starch and the ease with which the starch granules are disintegrated, whereas higher setback values are usually correlated with the amylose content of the starch (Copeland *et al.* 2009).



A



B



C D

Figure -2 - Scanning electron photomicrographs of little millet starch

CONCLUSION

The investigation was carried out with an aim of standardizing the isolation and characterization of starch in finger and little millets. The results indicate that modification of finger millet starch change the characteristics of starch. Swelling power of finger millet was increased with modifications. Little millet starch showed slow water absorption capacity than finger millet. Both millet starches showed comparatively lower breakdown, final viscosity and setback while finger millet starch showed higher values of breakdown, final viscosity and setback. Among the millets, Finger millet, a low cost, easily available and rich source of starch could thus serve as a source of modified starches. These could be exploited not only from the commercial angle but also to understand the behavior of starch for the future. These experimental results would provide useful information for consumers and food industries making use of millet starches.

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